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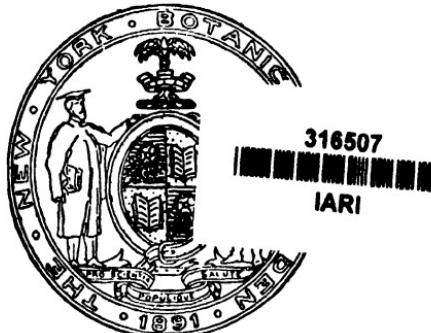


# MYCOLOGIA

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Volume XXIII, 1931

WITH 55 PLATES AND 73 FIGURES



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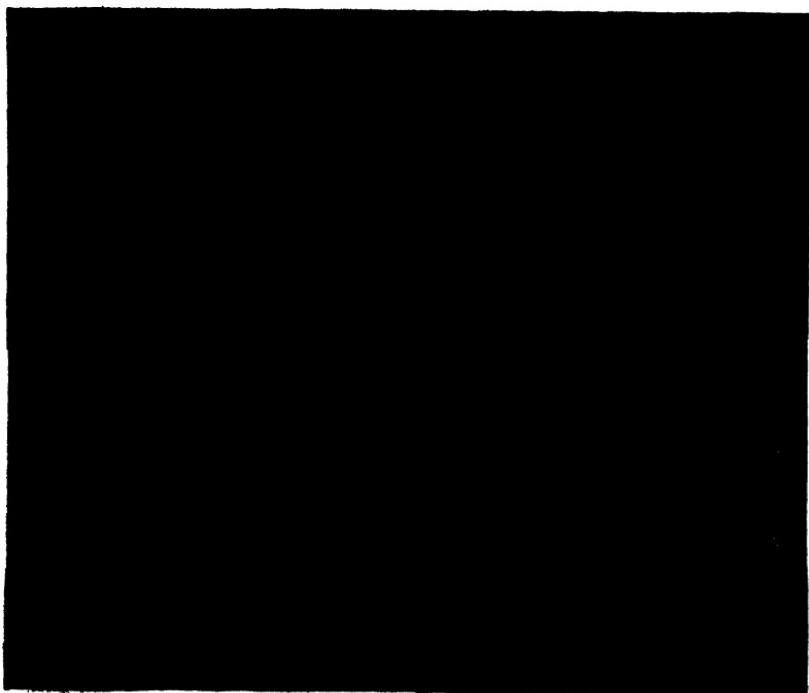
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ALBINISTIC AND TYPICAL NEUROSPORA SITOPHILA

# MYCOLOGIA

VOL. XXIII JANUARY-FEBRUARY, 1931

No. 1

## INHERITANCE OF THE ALBINISTIC NON- CONIDIAL CHARACTERS IN INTER- SPECIFIC HYBRIDS IN NEUROSPORA\*

B. O. DODGE

(WITH PLATES 1-7)

When an albinistic race of *Neurospora sitophila* is crossed with a conidial race of the species (PLATE 1), four of the spores from an F<sub>1</sub> ascus will give rise to albinistic mycelia and the other four will develop mycelia with typical monilioid conidia (PLATE 2). If these eight mycelia, which are the progeny of a single zygote "mother-cell," are mated in all possible combinations, four of the cultures will give rise to perithecia which produce only albinistic ascospores, eight cultures will mature asci heterozygous for conidia, and four cultures will produce only typical ascospores whose mycelia will bear monilioid conidia, illustrating Mendelian segregation in a very striking manner (3). Continuing from this point, the present paper records the results of experiments in crossing an albinistic race of *N. sitophila*, not only with a race morphologically indistinguishable from *N. tetrasperma*, which has four-spored asci, but also with *N. crassa*, another species with eight-spored asci. In the course of this work certain hermaphroditic hybrid segregate strains were developed which were

\* Presented by invitation before the section for Mycology and Plant Pathology of the Fifth International Botanical Congress held in Cambridge, England, August, 1930.

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more or less self-incompatible, but which crossed freely with the unisexual tester strain. Other fruit bodies which appear to be hybrids between two strains, each of which was fully capable of producing ascocarps by itself, were also obtained illustrating unique cases of a preference for cross-fertilization over selfing. These races will also be described.

Previous contributions on the morphology and cytology of species of *Neurospora* and interspecific hybrids are referred to in the paper cited above, and they need not be reviewed again, except to point out that when typical *sitophila* was crossed with typical *tetrasperma* (8) the segregations of factors for four-spored and eight-spored asci were certainly not according to any simple Mendelian formula. Allen (1, 2) crossed non-tufted races of *Sphaerocarpos Donnellii* with tufted races, and tufted with tufted races, and showed that segregations for the tufted character are not as clear cut as would be expected in simple Mendelian inheritance.

Since one of the parents used in making the interspecific crosses to be described was an albinistic race of *sitophila*, a race which produces a fluffy aerial growth and which seems to be sterile as to production of monilioid conidia, it would not be unexpected to find the results still further complicated if, instead of selecting a typical conidial strain of *N. tetrasperma* as the other parent, a four-spored segregate, no. 507, which produces only a scanty aerial growth with only a few conidia were chosen. When this was done, the results turned out to be even more irregular, conflicting and difficult to explain than was expected. On the other hand, crosses between albinistic non-conidial *sitophila* and typical conidial *crassa*, being crosses between species both of which have eight-spored asci, illustrate again rather simple Mendelian segregation. Only a comparatively few matings were studied and they will be described first, although the results obtained by crossing albinistic *sitophila* with the four-spored segregate no. 507 should be of far greater interest from a genetic standpoint.

*Neurospora crassa* is crossed with *N. tetrasperma* with great difficulty, and  $f_1$  ascospores are seldom matured in cultures of such matings. Some strains are more fertile when mated than

others, so that by mating several different sets of ascosporic strains one may be certain of finding a combination that will eventually mature ascospores. The perithecia, asci and ascospores of *N. crassa* are much larger than are those structures of *N. sitophila*. Nevertheless, fertile hybrids between typical strains of these two species have also been produced on several occasions. Segregations of the factors for sex and for conidia are the only ones which will be considered in the crosses between albinistic *sitophila* and typical conidial *crassa*.

#### ALBINISTIC *Neurospora sitophila* X TYPICAL *N. crassa*

Matings were made in test tube cultures on corn meal agar between mycelium no. 42a, a typical sex A race of *N. crassa*, and no. 56(2 X 6)a.2, which is an albinistic sex B race of *N. sitophila* (3, p. 18) and one of the progeny from a mating of two albinistic races. Each of the cultures finally developed a few perithecia and after about six weeks some ascospores were discharged. No extended observations were made as to the nature of the  $F_1$  asci and their spores due to other interests at the time. Six months later, surface sowings of the  $f_1$  ascospores were made in the usual way, and after heating the plates to kill any conidia present and to stimulate ascospore germination, it was found at the end of four hours that practically every one of the mature ascospores had germinated. Forty-four germinating spores which were in a position to be readily isolated were transferred to fresh agar plates where they were allowed to grow until the tip ends of the germ tubes were long enough to be cut off and transferred to tubes without including the original bit of agar bearing the ascospore itself and any possible conidia.

In this way forty-four cultures of single ascospore  $f_1$  haplotypes were obtained. At the end of seven days it was clear that three or four different types of mycelia were developing. Haplotypes 11, 15, 20, 23, 30 and 41 were albinistic, with profuse, fluffy, whitish, aerial growth and no conidia. Mycelia 40 and 44 appeared to be albinistic, yet a few conidia which remained attached for a long time were formed. Mycelia 8, 27 and 33 tended to be albinistic, but developed a rather scanty growth of pale aerial hyphae with only a few conidia. Of the other thirty-three

mycelia, some developed conidia quickly and profusely and others slowly, but finally all resembled very much typical cultures of the *sitophila* parent. Just why only six out of the forty-four mycelia were strictly albinistic and sterile as to conidia, and five others were really intermediates, is not clear. The albinistic haplonts which produced no conidia were back-crossed with stock tester strains, sex A and sex B of the conidial parent, *N. crassa*. Mycelia 11, 20, 23, 30 and 41 proved to be of sex A, and no. 15 was of sex B class. The other haplonts were not tested as to their sex.

The test culture in which the f<sub>1</sub> albinistic haplont 20 was grown with typical *crassa*, sex B, matured ascospores first. These back-cross asci were very long and slender, resembling the *crassa* parent species in this respect. The ascospores were fairly uniform in size, perhaps somewhat smaller, if anything, than are those of *N. crassa*. Several asci were selected and their spores isolated one by one so that the position of each spore in the ascus was known. The characteristics of the haplonts obtained are given below.

*Crassa* × (Non-conidial *sitophila* × *crassa*)<sub>20</sub>

ASCUS A. Ascus A was isolated and left on the surface of the agar still containing its spores. It was found on the following morning that seven of the spores had germinated without heating, a rather unusual occurrence. Tip ends of long germ tubes which could be traced back to individual spores with some degree of certainty were cut off and transferred to tubes. Mycelia 1 and 2 proved to be albinistic and of sex B. Mycelia 3 and 4 produced a profusion of conidia and were proved to be of sex A. It was not possible to be certain as to the identity of the three mycelia which came from spores in the lower end of the ascus. One was albinistic and of sex A. The other two were typical conidial mycelia and were of sex B. Although there exists some uncertainty as to the position of the ascospores giving rise to the particular tips of germ tubes transferred, it is clear that segregations of factors for conidia and for sex had occurred, resulting in ascospores of four types. The spindles of the first and second nuclear divisions in the ascus of *N. crassa* and *N. sitophila* are longitudinal. It may be assumed that the same orientation obtains in the long

slender ascii of their hybrids. It is very likely, therefore, that both sets of factors were segregated in the second division, and independently, since albinistic mycelia 1 and 8 were of opposite sex. An example of such a type was not found in the experiments reported previously (3). These experiments where albinistic and typical strains of *N. sitophila* were crossed have since been repeated and the results will be given later in this paper.

ASCI B AND B'. The spores from ascus B had been carefully isolated one by one. Cultures were obtained from spores 1, 5, 6, 7 and 8. Mycelium no. 1 was typical in that it quickly developed many pale orange-colored conidia. It proved to be of sex B. Mycelia 5, 6, 7 and 8 were albinistic and were all of the same sex, A. The factors for conidia, as well as those for sex, segregated in the first nuclear division. Although these four albinistic haplonts produced no monilioid conidia, nos. 5 and 6 were of a different type from that of 7 and 8. Their aerial growth was more open and fleecy. The aerial growth of 7 and 8 was rather compact, developing a pinkish ridge at the upper end of the agar slant. If this represents a third character which segregated in the second nuclear division, it could not readily show in case of mycelia like no. 1 because of the profusion of colored conidia. Old cultures of conidial mycelia from ascus D do show decided differences, however. Several transfers were made from cultures 5, 6, 7 and 8, yet the same differences between the pairs 5-6 and 7-8 were maintained. These particular mycelia all being of the same sex, of course, will not mate with each other. By crossing these strains with other albinistic strains it can be determined whether the differences noted are genotypic. Only four spores from ascus B' germinated. Segregation of both sets of factors occurred in the first nuclear division in the ascus.

ASCUS D. Mycelia 1, 2, 3 and 4 were typical, producing many conidia. They were all of sex A. Cultures of these four clons examined several weeks later showed differences which parallel those mentioned in connection with ascus B. Again both sets of factors separated in the first nuclear division, nos. 5 and 6 being albinistic and sex B.

ASCUS D'. Mycelia were obtained from spores 3, 6, 7 and 8. Mycelium 3 which was of sex B was of the albinistic type of aerial

growth, yet a few conidia were formed in an abnormal way. This type is indicated in table I by the sign  $\pm$ . It will be discussed briefly in a later paragraph. Mycelia 6, 7 and 8 produced an abundance of monilioid conidia. They were sex A; segregation of both sets of factors in the first nuclear division, but that for conidial factors was incomplete. Ascus L showed the same type of abnormality.

ASCUS I.. Mycelia were obtained from spores 1, 2, 3 and 4. They were all albinistic and again we have the type which produces some abnormal conidia, the same as no. 3 in ascus D'. The sex factors segregated in the second nuclear division.

ASCUS M. Mycelia 1, 2 and 7, 8 were non-conidial and sex B. Mycelia 3, 4, 5 and 6 were typical conidial and sex A. Both sets of factors separated in the second division, yet only two kinds of spores were formed. This type of ascus should be compared with ascus A where four different kinds of spores were produced with both sets of factors segregating in the second division.

ASCUS N. Mycelia were obtained from spores 1 to 5. Mycelia 1 and 2 were albinistic; 3, 4 and 5 were conidial; nos. 1, 2, 3 and 5 proved to be sex A, and no. 4 sex B. Evidently some slip occurred in isolating spores 4 and 5. If these two are transposed, it would mean a segregation of the sex factors in the first division and those for conidia in the second.

ASCI O, P AND Q. These three asci belong to the type showing segregation of factors for conidia and factors for sex in the first division. Culture 3 from ascus O is one of the albinistic intermediates.

The data dealing with the various asci just discussed are given in table I.

Even though only a few asci were studied, it is evident that the factors controlling the development of the albinistic non-conidial type of aerial growth, as contrasted with typical conidial mycelium, segregate during the divisions of the fusion nucleus of these interspecific hybrid asci. The fact is also brought out that both sets of factors may segregate together in either the first or second nuclear division in the ascus. Since we are considering only the factors for sex and those for conidia, only two kinds of ascospores were formed in ascus B. The difference between mycelia 5, 6

TABLE I  
SEGREGATION OF THE FACTORS FOR SEX AND FOR CONIDIA IN BACK-CROSS ASCI (ALBINISTIC NON-CONIDIAL *sitophila* X CONIDIAL *crassa*)  
*X crassa*

The first half (S) of each double column shows the sex, A or B, of the particular haplonts which were obtained by germinating spores from that ascus. The second half (C) of each double column shows the production (+ C) or lack of production (- C) of conidia by individual haplonts; albinistic mycelia which produce a few abnormal conidia are indicated by the sign ±. Spores that did not germinate are enclosed ( ).

Ascus No.	A		B		B'		C		D		D'		L		M		N*		O		P		Q			
	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C		
1	B	-C	B	+C	(B	-C)	B	-C	A	+C	(B	-C)	B	±C	B	-C	A	-C	(A	+C)	A	+C	(A	-C)		
2	B	-C	(B	+C)	(B	-C)	B	-C	A	+C	(B	-C)	B	±C	B	-C	A	-C	A	-C	A	+C	(A	-C)		
3	A	+C	(B	+C)	B	-C	B	-C	A	+C	B	±C	A	±C	A	+C	A	±C	A	+C	A	+C	(A	-C)		
4	A	+C	(B	+C)	(B	-C)	A	+C	(B	-C)	A	±C	(B	-C)	A	±C	A	+C	B	+C	(A	±C)	A	+C	(A	-C)
5	B	+C	A	-C	(A	+C)	(A	+C)	B	-C	(A	+C)	(B	+C)	A	+C	A	+C	B	+C	B	-C	B	+C	B	+C
6	B	+C	A	-C	A	+C	(A	+C)	B	-C	A	+C	(B	+C)	A	+C	(B	+C)	B	+C	B	-C	B	+C	B	+C
7	A	-C	A	-C	A	+C	(A	+C)	(B	-C)	A	+C	(A	+C)	B	-C	(B	-C)	B	+C	(B	-C)	B	+C	(B	-C)
8	(A	-C)	A	-C	A	+C	(A	+C)	(B	-C)	A	+C	(A	-C)	B	-C	(B	-C)	B	+C	(B	-C)	B	+C	(B	-C)

\* Spores nos. 4 and 5 evidently became misplaced.

and 7, 8 is such as to suggest that there were, after all, four genotypically different kinds of spores in this ascus.

It has been proved (6) that the sex factors very commonly segregate in the first nuclear division in the ascus of typical *N. crassa*. The factors for sex as well as those for conidia often separate in the second division in the ascus of *N. sitophila* (3), but in no case was it found that both sets separated together in the same division. If the segregations are by chance, such types should be met with. The results of further experiments given below show that such is the case.

#### ALBINISTIC NON-CONIDIAL $\times$ TYPICAL CONIDIAL *sitophila*

The Moreaus (12) have recently arrayed the evidence to prove that in such ascomycetes as *Sphaerotilisca*, *Pyronema* and *Neurospora* the sex organs do not function as such in fertilization, and Gwynne-Vaughan and her associates (10, 11) have put forward tentatively the theory of nutritive heterothallism. On the basis of either theory it would be difficult to explain the inheritance of the albinistic character in such a regular fashion, and we ought to find in crosses between albinistic and typical races of *N. sitophila* certain perithecia, or at least certain asci, in which the spores would be either all albinistic or all typical. Partly with this in mind, further experiments with such crosses were carried out and the nature of the spores in several asci from three different perithecia was studied. Strain 56.2, an albinistic strain, sex B, was mated with the typical strain 56.8, sex A (3, p. 23). The main purpose being to determine whether each ascus produced some albinistic spores, the exact position of the spores was not important and was not usually recorded. The types of spores obtained from only three of the eleven asci analyzed are noted.

ASCUS A. The eight spores were isolated in order and a culture obtained from each spore. Mycelia 1, 2 and 7, 8 were albinistic and of sex A. Mycelia 3, 4 and 5, 6 were typical and of sex B. Only two different kinds of spores were developed in this ascus. Clearly the factors for conidia and those for sex were segregated in the second division. In each case the +C factor for conidia went with sex B, so that the spores were arranged in the ascus:

two, four, two, with respect to their sex as well as to their conidial factors.

Ascus L. All eight spores germinated; clons 1, 2 and 5, 6 were proved to be conidial and sex A; 3, 4 and 7, 8 were albinistic non-conidial and sex B. Again both sets of factors segregated in the second division, giving only two types of spores, but in this ascus the spores alternated two and two for both sets of factors.

In ascus H both sets of factors segregated in the first nuclear division. The types obtained from the other asci were either the same as those previously described (3) or it was not possible to determine which divisions were the seat of segregations because the positions of the spores were not recorded. If some of these asci developed from the matings between conidial and non-conidial clons are homozygous for conidia, such have not been found. This would indicate that in the production of ascocarps by mating clons of opposite sex, both clons contribute nuclear elements which in some way come together and are represented by the fusion nucleus in the ascus. The results presented above further prove that segregations of factors for conidia and factors for sex segregate independently, either together in the first or the second nuclear division in the ascus, or one set may segregate in the first division and the other set in the second. As yet no evidence has been found of a segregation in the third division.

#### INTERSPECIFIC HYBRIDS

In crossing *Neurospora sitophila* with *N. tetrasperma* (8), it will be remembered that a species regularly having 8-spored ascii was crossed with one normally with 4-spored ascii. The spores of the former are unisexual, those of the latter are bisexual. The  $F_1$  ascus is long and slender like that of the *sitophila* parent. Eight spores are usually delimited and the ones that mature are about the size of spores of *N. sitophila* or perhaps a little larger. Mycelia from sixty  $f_1$  ascospores were mated in back-cross with each parent species. In no case were ascii from such back-cross perithecia homozygous for 4-sporedness as contrasted with 8-sporedness, which were the characters considered. By selecting and again back-crossing with *N. tetrasperma* a segregate no. 209 was obtained which when again back-crossed gave perithecia

with mostly 4-spored ascii. The process has since been continued to obtain a unisexual clon 507 which is indistinguishable from unisexual strains of the *tetrasperma* parent, except that for some reason, like 209, it produces a very scanty aerial growth and very few conidia on corn meal agar. If, then, one crosses clon 56.2, the albinistic non-conidial *sitophila*, with 507, he will be making practically interspecific crosses, and in addition have an experiment comparable to Allen's (1, 2), especially if he had crossed non-tufted with little-tufted clons.

NON-CONIDIAL *sitophila*, CLON 56.2 X WEAKLY-CONIDIAL  
"tetrasperma," CLON 507

Clon 56.2 was mated in several tube and plate cultures with no. 507. About six weeks later some spores began to mature. Crushed mounts showed that  $F_1$  ascii were commonly 8-spored and there was the same large percentage of abortion and slow ripening noted in the first experiments with crosses between typical *sitophila* and typical *tetrasperma* (8). Following the usual methods, cultures of seventy-nine mycelia were obtained from  $f_1$  ascospores selected at random. These cultures on corn meal agar were examined thoroughly for production of conidia. Without going into details, it may be said that every mycelium that did not plainly show conidia was repeatedly grown on dextrose agar, potato dextrose agar, white bread, rye bread, corn meal mush and wheat bran mixed, and potato plugs. Such media tend to induce luxuriant aerial growth, but unless a mycelium produces some few conidia on plain corn meal agar, conidia will not be found on these other media.

The following mycelia produced a fluffy aerial growth and quantities of monilioid conidia like typical strains of *N. sitophila*: nos. 4, 8, 9, 14, 35, 36, 39, 40, 43, 48, 56, 59, 61, 68, 69, 73, 75, a total of seventeen, or 21.5 per cent. Certain mycelia produced a scanty growth with few conidia like that of the parent 507. They were as follows: nos. 23, 37, 41, 42, 45, 63, 65, 66, 74 and 80, a total of ten, or 12.6 per cent. The following mycelia were of the albinistic non-conidial type resembling the parent clon 56.2: nos. 1, 2, 5, 6, 10, 12, 13, 15, 19, 20, 21, 22, 26, 27, 29, 31, 32, 33, 34, 46, 47, 52, 55, 58, 60, 64, 67, 71, 76 and 77, a total of thirty,

or about 38 per cent. Those mycelia making scanty aërial growth like 507, but without conidia, were the following: nos. 3, 7, 11, 16, 17, 18, 24, 25, 28, 38, 49, 50, 51, 53, 54, 57, 62, 70, 72, 78, 79 and 81, a total of twenty-two, or 27.8 per cent. In all, fifty-two, or 66 per cent of the seventy-nine mycelia studied, were non-conidial, and twenty-seven, or 34 per cent, were conidial; forty-seven, or 59 per cent, gave good aërial growth, while thirty-two, or 41 per cent, made scanty aërial growth.

It is to be regretted that all eight spores in  $F_1$  ascii so seldom mature, making it practically necessary to choose spores at random. Here, just as was the case where albinistic "non-conidial" *sitophila* was crossed with typical conidial *crassa*, only in the reverse, some element has entered to upset the exact 50:50 ratio of conidial to non-conidial mycelia. Only a small percentage of the  $f_1$  spores actually ever mature, and of these not all germinate. The character "scanty growth," coming from the parent 507, which normally ripens quickly, was carried by only 41 per cent of the spores, so that there seems to be no connection between early ripening and scanty growth. This work must be repeated using as one parent a strain of *N. tetrasperma* which produces an abundance of conidia, instead of clon 507.

#### SEX OF THE $F_1$ CLONS

Sixty-five of the  $f_1$  mycelia were tested for their sex. The following were sex A: nos. 3, 5, 6, 11, 19, 20, 23, 24, 25, 27, 31, 32, 35, 36, 38, 39, 40, 41, 42, 43, 44, 46, 47, 53, 58, 60, 65, 69, 70, 73, 74, 76, 78, 79, 80, 81, a total of thirty-six. The following were sex B: nos. 1, 2, 4, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 21, 22, 26, 29, 33, 34, 37, 45, 48, 54, 55, 64, 67, 71, 75, a total of twenty-nine. Originally nos. 1 to 48, except nos. 28 and 30, which were lost, and five others were tested. The ratio of the two sexes stood 24:27. Several months later thirteen more cultures were tested. They were all sex A except no. 75. The germinating spores, as stated above, were originally selected at random, and the cultures were given their numbers at once, yet beginning with no. 38 we have seven consecutive mycelia all of the same sex A. From no. 70 to 81 the mycelia were mostly sex A; nos. 12 to 18 were sex B. This shows that random selections may sometimes give some peculiar results.

The  $F_1$  ascus is at least diploid and anything like "dominance," which is an attribute manifested by the sporophyte generation, could best be expressed in the form taken by the ascus, which is the only sporophytic structure with definite characters. In addition to being long and slender, the  $F_1$  ascus is 8-spored, and, like the asci of the *sitophila* parent, the ascospores are unisexual, so that  $f_1$  mycelia are "heterothallic." Not one of these mycelia was found to be hermaphroditic. Considering merely the number of spores in the ascus, 8-sporedness is, in a sense, "dominant."

#### FIRST GENERATION BACK-CROSSES

Fifteen  $f_1$  mycelia, selected because they were albinistic non-conidial, were among those tested for their sex. Twelve were sex B and only three were sex A. Clons 29 and 64, sex B, and clon 47, sex A, were chosen to mate with stock strains of *N. tetrasperma*,  $S_6$  and  $S_1$  respectively, in a series of selections to obtain homothallic albinistic non-conidial clons which would develop perithecia with 4-spored asci like *N. tetrasperma*. The mycelia derived from mating 29 with  $S_6$  will be referred to as set I, those from mating 47 with  $S_1$  comprise set II, and those from  $64 \times S_6$  are included in set III.

SET I. The mating  $(507 \times 56.2)_{29} \times S_6$  developed ascospores first. Being virtually first generation back-crosses, the asci contained variable numbers of spores, mostly from two to six, varying from 20 to 60  $\mu$  or more in length. All spores cut out seemed to mature. The larger spores must have contained several nuclei, probably in this case differing genotypically. Sowings were made and a number of germinating small spores were selected because, being very small, they must be unisexual. The inheritance represented in a giant spore can be determined by plating out the conidia from its mycelium. The purpose of these experiments, however, was principally to obtain albinistic non-conidial haplonts. Their mycelia differed greatly as to color, types of aerial growth, and production of conidia. These differences were heightened for some and decreased for others as the cultures aged (PLATE 4). The notes given below were made when the cultures were about ten days old, with modifications later as necessary.

1. Non-conidial; albinistic, whitish fluffy aërial growth.
2. Conidial; at first whitish to light brownish aërial growth, then turning a beautiful bright saffron pink. Later transfers did not show such striking colorations.
3. Non-conidial; brownish fluffy growth, later fading to whitish.
4. Non-conidial; pale light brownish fluffy growth.
5. Non-conidial, and the same type as no. 4.
6. Conidial; light brownish aërial hyphae, then light orange-colored like *N. sitophila*.
7. Non-conidial; whitish, fleecy and albinistic growth.
8. Non-conidial; somewhat albinistic, then slightly orange to brownish growth.
9. Non-conidial; pinkish felty surface growth.
10. Non-conidial; scanty aërial growth.
11. Conidial; rather scanty, felty aërial growth.
12. Non-conidial; at first pale fluffy aërial growth, then orange-brownish.
13. Non-conidial; scanty growth.

Eight of the non-conidial mycelia were tested out for their sex; nos. 1 and 13 were sex A and 3, 4, 5, 7, 8 and 9 were sex B.

SET II.  $(507 \times 56.2)_{47} \times S_1$ . Mycelium 47, being sex A, was mated with stock clon  $S_1$ , sex B of *N. tetrasperma*. Twenty-one mycelia were obtained from the unisexual ascospores. The characters of the first twelve are given below (see also PLATE 4). The other nine, for some reason, did not show much aërial growth and were not studied fully.

1. Conidial; light orange-colored aërial growth, turning to reddish orange later; conidia much more abundant than most strains of *N. tetrasperma*.
2. Non-conidial; pale pinkish aërial growth, tending to albinistic.
3. Conidial; not very heavy orange-colored growth.
4. Conidial; pinkish-orange, conidia persistent, later turning darker orange-colored to brownish.
5. Non-conidial; a typical albinistic growth.
6. Non-conidial; very scanty aërial growth.
7. Non-conidial; at first a scanty aërial growth, later becoming fluffy to felty.
8. Conidial; scanty aërial growth, becoming felty.
9. Conidial; rather pale orange-colored, like Arl. 10 strain of *N. sitophila*. Good aërial growth.
10. Conidial; pale fluffy growth with many conidia later.
11. Non-conidial; scanty aërial growth.
12. Non-conidial; very little aërial growth.

Again one notes that when a mycelium produces conidia, it does so with vastly greater abundance than does either the original conidial parent, 507, or the immediate back-cross parent  $S_1$ .

Clons 7, 12 and 20 were proved to be of sex A and 1, 2 and 5 were of sex B. The others were not tested for sex.

SET III. ( $507 \times 56.2_{64}$ )  $\times S_6$ . Twelve mycelia were obtained as usual by germinating the small unisexual ascospores from this mating. Their characters are given here to further prove that, so far as types of aërial growth, production of conidia and coloration are concerned, segregations were not such as to produce only the two kinds represented by their immediate parentage. No doubt, however, if one analyzed the total inheritance represented by the eight nuclei from any particular ascus he would find for any one character four nuclei of one kind and four of the contrasted character.

1. Non-conidial; very little fluffy growth, but a rather compact, pinkish ridge of growth at the top of the agar slant.
2. Non-conidial; same as no. 1.
3. Non-conidial; growth like no. 1.
4. Conidial; bright orange-colored growth, conidia like *N. sitophila*.
5. Conidial; great abundance of orange-colored conidia.
6. Non-conidial; very scanty aërial growth.
7. Conidial; rather pale faded orange-colored growth.
8. Non-conidial; very scanty growth.
9. Non-conidial; much like no. 8.
10. Non-conidial; somewhat albinistic but rather limited aërial growth.
11. Non-conidial; little aërial growth.
12. Non-conidial; like no. 11.

Tested for their sex, nos. 1, 8, 9, 10, 11 and 12 were of sex A type, and 2, 3, 4 and 6 were sex B. The other two were not tested.

Thirty-seven haplonts included in sets I, II and III were examined very carefully for production of conidia. Twelve, only, produced conidia. Without counting the nine apparently non-conidial mycelia discarded from set II, only 32 per cent were conidial. Twenty-four mycelia were tested for their sex. Eleven were sex A and thirteen were sex B. As noted previously, several of these mycelia produced conidia in amounts greatly in excess of what either no. 507 or  $S_6$  ever produces on the same medium. Nowhere in any strains of either species has such a bright saffron pink and orange color as shown, for example by clon 2 in set I, been observed. This would be something entirely new if it continued to keep these colors in future transfers. For some reason, the few transfers made do not show such brilliant colora-

tions. Clon 1 in set II retains its deep colors in subsequent transfers.

PROGENY OF MATING  $\{(507 \times 56.2)_{29} \times S_6\}_8 \times S_6$

Comparing crushed mounts of perithecia from cultures comprising sets I, II and III, it was found that the culture in which  $S_6$  was mated with clon 8 of set I gave ascii fairly regularly 4-spored, but occasionally an ascus would have five or six spores. Several ascii containing mature spores were selected and their spores isolated one by one. All four spores in several ascii germinated. The characters of the mycelia derived from some of these ascii will be described first, beginning with the ascii which appeared to be fairly regular as to segregations of factors for sex and for conidia. Later a table will be given to include all ascii studied.

The peculiar orientation of the spindles in the three nuclear divisions in the 4-spored ascus of *N. tetrasperma* has already been described in some detail (7). A spore will ordinarily contain one nucleus of each sex when cut out, making it bisexual and its mycelium totipotent. It is only rarely, or about 1 per cent of the time, that both nuclei in a binucleate spore are of the same sex. Such a distribution of the nuclei is easily explained. If the sex factors segregate in the second nuclear division and, while the four reorganized nuclei are separating on their spindles, the two bearing factors for sex B move one way and the other two bearing sex A factors move in the opposite direction, then two nuclei, each containing sex A factors, are included in each of two spores. The other two spores would each be provided with two sex B nuclei. Irregularities in spindle orientation such as must be expected in hybrids could also enter and affect the position and thus the distribution of the nuclei which are included in particular spores.

Where possible in this work two sets of cultures were obtained from germinating spores; one by transferring tip ends of germ tubes and the other by transferring the ascospores with some of the remaining growth. As a spore begins to germinate, the nuclei divide rapidly so that the germ vesicles and their branches contain several nuclei each. Where the two original nuclei differ genotypically, one is never certain that in transferring a single

germ tube tip he gets all of the inheritance represented by the spore. This will be seen by referring to figures of nuclei in ascospores and conidia (9).

ASCUS G. The four spores, isolated in order, germinated. Mycelia nos. 3 and 4 appeared not to produce conidia and the cultures showed young perithecia on the third day. Nos. 1 and 2 were conidial and began to show perithecia on the fifth day. Later tests indicating that 3 and 4 produced few if any conidia, it became necessary to prove that both nuclei in spore no. 1 carried +C factors for conidia, and to check this up by proving the same thing for spore no. 2. Following the method previously described (9), twenty-nine mono-conidium cultures were obtained from mycelium 1G. Of these mycelia nineteen were bisexual and produced an abundance of perithecia. Ten mycelia, or 35 per cent, were unisexual, four of these being sex A and six sex B. When one plates out single conidia from normal *N. tetrasperma* he frequently gets as high as 35 per cent unisexual mycelia. Mycelia 6 and 29 produced quantities of orange-colored conidia; nos. 9, 12, 19 and 20 produced a scanty aerial growth and only small quantities of conidia. These haplonts all happened to be sex B. The other four were sex A and produced only a very few conidia, scarcely visible even with a hand lens. Just how many of these differences are merely phaenotypic or are connected with the sex of the mycelia is a question. The bisexual mycelia showed comparable differences in the amounts of conidia produced.

Twenty-five mono-conidium cultures were obtained by plating out conidia from mycelium no. 2G. Here again there were three kinds of mycelia as regards their sex and all likewise produced conidia. Fifteen mycelia were bisexual and ten were unisexual. Of the latter, four were of sex A and six were sex B. Clons 20 and 25, both sex B, developed fairly large quantities of orange-colored conidia. The other eight produced only a comparatively few. Since none of the twenty unisexual haplonts derived by plating out conidia borne on mycelia 1G and 2G failed to produce conidia and as the sexes A and B were both represented it is evident that there was a segregation of the factors for conidia in ascus G. The eight nuclei were so distributed that the four

carrying the +C factors for conidia were all included in spores 1G and 2G. With segregation of the sex factors in the first nuclear division and those for conidia in the second division one can readily see how such a distribution can come about if the spindles are oriented the same as they are in normal *N. tetrasperma*. During the second nuclear division the spindles lie as though in conjugate division and oblique to the long axis of the ascus. In segregating, the +C factors for conidia pass to the nucleus at the upper end of the spindle in each case. A third division in which the spindles are more or less transverse then occurs. This leaves the four nuclei carrying +C factors in the upper end of the ascus and they will all be included in spores nos. 1 and 2.

ASCUS O. The four spores germinating, two sets of cultures were readily obtained. Mycelia 1, 2 and 3 produced conidia, and perithecia quickly developed; no. 4 in both sets appeared to be non-conidial. The one derived from a "tip" transfer produced perithecia, but the culture from the ascospore remained sterile for a long time. Very likely a few conidia which were overlooked were produced by no. 4. A discussion of weakly bisexual mycelia will be taken up later on. Segregation of the factors for conidia and those for sex could have occurred in either the first or second nuclear division.

ASCUS Q. Each of the four mycelia produced conidia, and in due time matured perithecia, showing that each spore contained nuclei of both sexes and one nucleus of each carried the +C factor for conidia. Tests were not made to learn whether all four nuclei of either sex carried such factors.

ASCUS R. All four spores germinated and again two sets of cultures were obtained. The four mycelia in each set produced conidia. Nos. 1 and 2 were weakly bisexual, developing comparatively few ascocarps. Nos. 3 and 4 were strongly bisexual. A great number of ascocarps matured within ten days. Evidently the segregations for conidia were regular, a point easily proved by plating out the conidia from the four cultures, or by germinating the small unisexual ascospores. There was nothing in the appearance of any of the four cultures to indicate the hybrid nature of the ascocarps.

ASCUS D. Only spores nos. 1 and 2 germinated. Both mycelia produced conidia and perithecia appeared in both cultures, discharging ascospores freely on the eighth day.

It is clear from the foregoing account that each of the spores from the five asci described contained nuclei of both sexes, and that, with the exception of spores 3 and 4 from ascus G, the +C factor for conidia was carried by at least one nucleus in each spore. No attempt was made to induce conidial formation by mycelium 4 from ascus O. It may have been weakly conidial. Spores 1 and 2 in ascus G were the only ones in which both nuclei carried conidial factors. There was a suggestion that their mycelia produced more conidia than was the average for mycelia where only one of the two nuclei in the spore carried the +C factor. This would need much more confirmation, as there is apt to be much irregularity in the quantity of conidia produced by any mycelium under slightly different conditions.

Blakeslee, Burgeff and others, working with the Mucoraceae, have noted that occasionally a monosporic mycelium derived from a primary sporangium remains neutral, not reacting with either tester strain. Various explanations have been put forward to account for such "neutrals." Comparing the reactions of various mycelia, these authors have found all grades of fertility or strength of sexual reaction. Just such irregularities have been noted from time to time in testing clonal stocks of *Neurospora* species. Some of the results of crossings being described here would suggest that in addition to, or instead of, weak sexuality, we would have to consider the possibilities of self-sterilities and incompatibilities. For example, the mycelia derived from spores from asci J and L behaved so erratically in tests for their sexuality as to indicate at first very poor technique in making transfers, or contamination of cultures by mites or otherwise. Against such an assumption may be cited the fact that no perithecia have ever appeared in stock tester strains of each of three species where transfers are constantly being made and old cultures are often kept around the laboratory for several months. Some details of the culturing done in testing out mycelia derived from asci J and L are given below, even though it was thought best at one time to discard the whole series as merely illustrating poor tech-

nique. Results of later experiments, as will be seen, account fully for all the apparent irregularities here reported, and reveal some interesting characteristics of these interspecific hybrids.

Ascus J. All four spores germinated. Bits of agar each bearing a spore were transferred to another plate and placed in order at about equal distances around the periphery of the petri dish. After the germ tubes had grown out and branched, tip ends from each transplant were cut off and transferred for cultures in set I. The ascospores themselves, together with short pieces of attached growth, were transferred to tubes for set II. In each case there was left in the plate enough growth to represent each spore. Mycelia grew out meeting along four lines, forming four sectors. After about a week perithecia began to appear all over sector no. 4, and then later a few formed at the outer end of the line where mycelia 2 and 3 met, followed by a few scattered irregularly here and there over sector 3, especially adjacent to sector 4. Even after six weeks none appeared on sectors 1 and 2. In the meantime, two other plates were inoculated, one with the inocula in the same relative positions as before, the other with nos. 2 and 3 transposed. Not a single ascocarp developed in the first culture. A few did finally form in sector 3 in the other culture. Mycelia 2 and 4 were planted in another plate culture. Some few perithecia finally formed at one point where the no. 4 transplant was placed.

Turning to the tube cultures, we find that all four mycelia in both sets I and II developed some conidia. The original cultures in set II kept for seven months never developed any ascocarps, and so long did all the cultures in both sets remain free of perithecia that it was thought here was a clear cut case of segregation for sex in such a way as to throw two nuclei of the same sex in each spore. This would mean that by mating mycelia nos. 1 to 4 in all possible combinations, one could readily determine which spores had contained two nuclei of sex B and which two nuclei of sex A. The first plate culture had strongly suggested that mycelium 4 must be sex A, because in *N. tetrasperma* matings of  $S_1$  and  $S_6$ , perithecia always form on  $S_6$ , which is sex A, and not on  $S_1$ , which is sex B (PLATE 7, FIG. 17).

Ordinarily in making tests for sex, perithecia will make their

appearance within from three to six days. If the strain in question does not react with either tester strain within this time, another test is made, but the former cultures are kept under observance until the question is settled one way or the other.

The following combination cultures were made at various times. If, even after several months, perithecia ever appeared in any culture, this is indicated by the sign "+"." The sign "-" indicates that no perithecia developed. Tester strains  $S_6$  and  $S_1$  are, as noted above, the stock unisexual tester strains of *N. tetrasperma*.

#### TEST I

$1J \times S_1 = -;$	$1J \times 2J = -;$	$1J \times S_6 = +$
$2J \times S_1 = -;$	$1J \times 3J = +;$	$2J \times S_6 = +$
$3J \times S_1 = +;$	$1J \times 4J = +;$	$3J \times S_6 = (-) +$
$4J \times S_1 = +;$		$4J \times S_6 = (-) +$

Since cultures  $3J \times S_6$  and  $4J \times S_6$  did not show perithecia at first, the results were thought to prove conclusively that 1J and 2J were of sex B and 3J and 4J were of sex A. Another test was made when, later, perithecia did show up in the tubes where 3J and 4J were grown with  $S_6$ .

#### TEST II

$1J \times S_1 = -;$	$1J \times S_6 = +$
$2J \times S_1 = -;$	$2J \times S_6 = +$
$3J \times S_1 = +;$	$3J \times S_6 = -$
$4J \times S_1 = -;$	$4J \times S_6 = +$

Since the second test shows 1, 2 and 4 as sex B and no. 3 only as sex A, a third test was made as follows:

#### TEST III

$1J \times S_1 = -;$	$1J \times S_6 = +;$	$1J \times 2J = -;$	$2J \times 4J = -$
$2J \times S_1 = -;$	$2J \times S_6 = +;$	$1J \times 3J = -;$	$3J \times 4J = -$
$3J \times S_1 = +;$	$3J \times S_6 = +;$	$1J \times 4J = -;$	
$4J \times S_1 = -;$	$4J \times S_6 = +;$	$2J \times 3J = -;$	

With the exception of  $3J \times S_1$ , all combinations in this test showed that the four mycelia were now of sex B. By this time it was noted that cultures 2 and 4 of the originals in set I showed a few perithecia, and one of the sub-cultures from 3J also bore perithecia. This was about three months after the first cultures were obtained. The four cultures in set II derived from asco-

spores were still devoid of any signs of perithecia. Transfers were made from this set and new tests made as follows:

TEST IV

$1J \times S_1 = -;$	$1J \times 3J = -$
$2J \times S_1 = -;$	$1J \times 4J = -$
$3J \times S_1 = -;$	$2J \times 3J = +$ (one perithecium)
$4J \times S_1 = -;$	$2J \times 4J = +$ (one perithecium)

The results were again irregular.

While testing mycelia from ascus J, the progeny from ascus L were also under investigation.

Ascus L. Three sets of cultures, I, II and III, were obtained from the four spores from ascus L. A few conidia were developed in each culture, but only a very scanty aerial growth was made on corn-meal agar. The first tests for the sexuality of the mycelia were made in petri dish cultures where the four were grown together. Placed at equal distances around the periphery, the four mycelia met along four lines, forming four sectors, as is usual, but at the end of ten days no fruit bodies had formed in any one of the four different plates. This indicated that all four mycelia were of the same sex. These cultures were discarded and further tests made in tube cultures, as follows:

$1L \times S_1 = -$ (+);	$1L \times S_6 = +$
$2L \times S_1 = -;$	$2L \times S_6 = +$
$3L \times S_1 = -;$	$3L \times S_6 = +$
$4L \times S_1 = -;$	$4L \times S_6 = +$

Since perithecia formed quickly in all combinations with  $S_6$ , it was clear that the four mycelia were of the same sex, A, confirming the petri dish tests. The combination  $1L \times S_1$  did not show perithecia until about six weeks later. Hoping to prove that here was a case where all of the eight nuclei in an ascus were of the same sex, the mycelia were grown together in all possible combinations. The results were again negative, further proving that the four mycelia were sex A. The original cultures in sets I, II and III, not showing any perithecia after being under observation for about two weeks, had been set aside and sub-cultures used for testing. Now, after several more weeks it was found that nos. 1 and 4 in each set showed a few mature ascocarps. Choosing 1 and 4 from among the sub-cultures showing no fruit bodies,

other tests were made, using various tester strains of *N. sitophila* and unisexual hybrid strains. All of these gave only proof that nos. 1L to 4L from the sub-cultures were of sex B.

With the idea that if each spore contained nuclei of both sexes, but that perhaps the sex A nucleus was acting in some incompatible capacity, attempts were made to separate the two by plating out the conidia. About twenty single conidium mycelia were obtained from 1L. None of these produced perithecia in culture. They were all proved to be sex B. Fourteen single conidium strains of 2L were tested against S<sub>1</sub>. No fruit bodies were formed. Several tested against S<sub>6</sub> gave perithecia. While

TABLE II

Ascus	Spore	Conidia	Sex	Remarks
A	1	+C	A + B	Scanty growth, weak sexuality.
	3	+C	A	Scanty aerial growth
	4	-C	A + B	Very strong sexuality; ripe ascospores within eight days.
B	3	+C	B	Only one spore germinated.
C	1	+C	B	Some few large sterile bodies
	3	+C	B	
	4	+C	A + B	Weak sexuality.
D	1	+C	A + B	Strong sexuality; matured ascospores within eight days.
	2	+C	A + B	Strong sexuality; matured ascospores within eight days.
E	2	+C	A	Culture produced one large sterile body.
F	1	-C	A(+B)	Strongly sex A; very weakly A + B.
	2	-C	A + B	Very weak sexuality.
	3	+C	A + B	Fairly strong sexuality.
	4	+C	B	
G	1	-C	A + B	Very fertile.
	2	-C	A + B	Very fertile.
	3	+C	A + B	Slow development but fairly fertile.
	4	+C	A + B	Slow development but fairly fertile.
H	1	+C	B	May have had two sex B nuclei.
	2	+C	B	Small spore.
	3	-C	A	Probably had two sex A nuclei.
	4	+C	A + B	Matured many ascocarps.
	5			Fifth spore small; did not germinate.
J	1	+C	B	See text for explanation.
	2	+C	B(+A)	See text for explanation.
	3	+C	A(+B)	See text for explanation.
	4	+C	B(+A)	See text for explanation.

TABLE II (Continued)

Ascus	Spore	Conidia	Sex	Remarks
K	1	+C	A + B	Very weakly bisexual; a few perithecia after several weeks.
	2	+C	A + B	
L	1	+C	B(+A)	Fairly strong sex B; slowly and weakly bisexual.
	2	+C	B	
	3	+C	B	
	4	+C	B(+A)	Fairly strong sex B; very slowly and weakly bisexual.
M	1	+C	A(+B)	Very slowly and weakly bisexual; strongly sex A.
	4	-C	B	
N	1	+C	A	Weakly bisexual; strongly sex A.
	2	+C	A(+B)	
	3	+C	A	
O	1	+C	A + B	Weakly bisexual. Conidia very few if any.
	2	+C	A + B	
	3	+C	A + B	
	4	-C	A + B	
P	1	+C	B(+A)	Approaches neutral, but was bisexual.
	2	+C	B(+A)	Approaches neutral, but was bisexual.
Q	1	+C	A + B	
	2	+C	A + B	
	3	+C	A + B	
	4	+C	A + B	
R	1	+C	A + B	Only a few perithecia.
	2	+C	A + B	Not very strong sexually.
	3	+C	A + B	Many perithecia.
	4	+C	A + B	Great abundance of perithecia.

mycelia 1L and 4L in the three sets of original cultures produced perithecia all other tests seemed to indicate that the mycelia were unisexual and reacted rather normally but not strongly as sex B.

The nature of the mycelia from nine other ascospores where not all of the spores germinated was also studied, but without extensive tests for their sexuality. Several mycelia gave promise at first of being neutrals, but all finally produced some perithecia either alone or in a mating with tester strains of one or the other sex. The results are given in the above table. Under the column headed "Sex," A + B means that the mycelium produced perithecia in culture. A(+B) or B(+A) means that ordinary tests

would show the mycelium to be unisexual, but when allowed to grow alone a long time in culture it would finally develop at least one or two good perithecia. Such mycelia will, for convenience, be referred to as "weakly bisexual."

It would be strange if there were not some nuclear abortion before spore delimitation in these hybrids. An ascus might contain four spores apparently of equal size, yet one of them have only one of the two nuclei which it would have had if the other nucleus had not aborted. Judging from what we know of nuclear behavior in the parent species *N. tetrasperma*, a uninucleate spore will be very small, because there is the companion uninucleate spore to require spore plasm for its growth also. With the one nucleus aborted the survivor of the pair is free to appropriate enough extra spore plasm to increase its size to that of the other spores with two nuclei. Such a condition could be readily proved if every nucleus carried +C factors for conidia. Should, however, one of the two nuclei in a spore be weakly, say, sex A, or in some way have taken on an incompatibility in relation to its natural mate nucleus, sex B, no perithecia would develop in a culture from the mycelium from this spore. Grown with some other unisexual sex A mycelium, perithecia would be formed. Grown with some other unisexual sex B mycelium, no fruit bodies would form because of antagonism between the normal sex B nuclei contained in each of the mycelia in the culture. The table does not show fully how irregular, inconsistent and inconclusive the results of the tests for sexuality really were. For this reason the work was repeated starting with fresh cultures of the mating  $\{S_6 \times (507 \times 56.2)_{29}\}_8 \times S_6$ . The progeny from each ascus studied will be briefly described. With the exception of ascus M', which had five spores, each ascus selected had only four spores, all about the same size, suggesting that each contained at its origin two nuclei of opposite sex.

Asci A', D' AND E'. The spores from each of these three asci all germinated, and their mycelia produced conidia and perithecia with no suggestion of irregularities. When incubated at 27° C. fruit bodies began to form about the fourth day.

Ascus B'. Cultures were obtained from spores 1, 2 and 3. Mycelia 1 and 2 produced conidia; no. 3 was apparently non-

conidial. No cultures on other media were made to test this out further. Perithecia did not begin to appear in cultures 1 and 3 until the seventh day; no. 2, failing to produce perithecia at this time, was thought to be unisexual. Tested against  $S_1$  it reacted strongly as sex A. This was further confirmed by the fact that no ascocarps formed in the culture where it was grown with  $S_6$ . At the end of a month perithecia began to form in the original culture. Later cultures on corn-meal agar to which had been added a little potato dextrose agar produced numbers of perithecia but only after considerable delay. This illustrates again how necessary it is in this work to keep the culture under observation for a long time; otherwise one may be misled into thinking the mycelium unisexual, not only because no ascocarps have been formed but also because of its strong reaction with the one or the other tester strains. Mycelium 2B' was in reality bisexual, being provided with nuclei of both sexes at the origin of the ascospore. Some element of infertility or incompatibility is present to prevent the full expression of the sex B nuclei until after a long time has elapsed.

ASCUS C'. The four mycelia derived from ascus C' all matured a few conidia. Nos. 3 and 4 developed perithecia on the fifth day. Culture 1 first showed ascocarps on the eighth day; otherwise it is strongly bisexual. Culture 2 and several subcultures from it have remained sterile for four months. There seems no likelihood of any perithecia forming at this late date. Tested several times against  $S_1$  it always reacts strongly as sex A. Grown with  $S_6$  no fruit bodies ever form. By every test made mycelium no. 2 was unisexual. The other three mycelia derived from the same ascus were bisexual. Degeneration of one of the eight nuclei before spore formation would account for this irregularity.

ASCUS F'. The four mycelia bore conidia; nos. 3 and 4 were strongly bisexual; nos. 1 and 2 produced only a few perithecia and these very slowly. Their reactions when the four mycelia were all grown together in a petri dish culture are suggestive. The sectors occupied by 3 and 4, being adjacent, covered half of the plate. This part was soon thickly dotted all over with ascocarps, while none had appeared on sectors 1 and 2 by the

eighth day. Tested in tube cultures against  $S_1$ , no. 2 reacted strongly as sex A. Cultured alone in tubes some few perithecia always develop in due time, however, showing the dual nature.

Ascus G'. Each of the four spores germinated. Mycelia 1, 2 and 4 produced many more conidia than is usual for these hybrids; no. 3 developed about the average quantity. A few perithecia were finally matured in cultures 1 and 2. No ascocarps were formed in cultures 3 and 4 during four months. The few tests made indicate that both 3 and 4 are of sex B; in which case each ascospore could have received only one sex B nucleus. It may be that we have here merely another example of self sterility or strong incompatibility. This would be difficult to prove unless the +C factors for conidia were distributed among the eight original nuclei in the ascus so that they would be carried by two nuclei of each sex. The production of microconidia by the albinistic strains of *N. sitophila* should not be forgotten (3). Should such asexual reproductive bodies be developed by the hybrid mycelia with which we are now dealing they could be isolated. Theoretically, in the case under discussion the microconidia could well be of sex A instead of sex B. Many of these mycelia appear at first to be unisexual in culture and then after several weeks or months slowly develop perithecia. This warrants caution in coming to hasty conclusions as regards their sex nature.

Ascus H'. One spore failed to germinate. The mycelium from no. 1 appears to be unisexual, sex B; nos. 2 and 3 proved to be bisexual. All three produced conidia. If further tests on other media likewise prove no. 1 to be unisexual, spore 4 should have been unisexual and sex A; whether its mycelium would have produced conidia involves too much speculation.

Ascus K'. Only spores nos. 1 and 2 germinated. Both mycelia were conidial. After about ten days a few ascocarps began to appear in culture 1. Culture 2 continued sterile for several days. Tested against  $S_1$ , both react strongly as sex A. Tested against  $S_6$  the cultures are either sterile or produce fewer perithecia than either one does by itself. Culture 1 is at first only weakly bisexual but finally after about two months many good perithecia develop.

Ascus M'. This is the first ascus with five spores from which the five mycelia have been analyzed. Some of the details will be given because they show clearly segregations for conidia and definite distribution of the nuclei carrying conidial factors and throw further light on the question of "weakly bisexual" mycelia. Spore 1 was slightly larger than normal. Spores 2 and 3 were very small spores, about  $22 \mu$  long. Spores 4 and 5 were of average size for bisexual spores. All the spores except no. 3 germinated within a few hours after they had been heated. It was not until a day later that no. 3 began to germinate. It was carefully transferred to a plate culture where the mycelium grew very slowly for two days; then it began to grow normally. This fact will be referred to again. Two sets of cultures were obtained, tip ends of germ tubes being transferred for set I and the remaining growth including the ascospores themselves for members of set II. Mycelia 1, 2, 4 and 5 developed conidia; no. 3 was grown on several kinds of media but in no case could any conidia be found. Cultures 1, 4, and 5 in both sets as well as in subcultures produced perithecia rather slowly but in some quantity. They would not be classed as strongly bisexual. Cultures 2 and 3 remained sterile. This was to be expected of the mycelia from the two small spores in a 5-spored ascus. In studying the cytology of asci of *N. tetrasperma* (7) nothing was found to indicate just why occasionally only one nucleus is included in each of two spores. One would naturally suspect some incompatible repulsion or weak sex attraction as responsible for the irregularity. No test has heretofore been made to ascertain whether the two small spores in such asci are of the same sex or not. It is of interest therefore to learn from the results of tests with our two mycelia that no. 2 is sex A, producing many perithecia in cultures with S<sub>1</sub>. Grown with S<sub>6</sub> no fruit bodies are formed. Mycelium 3 was proved in the same way to be sex B. Should there be any incompatibility relationship it did not show very strongly or conclusively when clon 2 was grown in culture with no. 3. Such combination cultures developed about the same number of ascocarps as did the bisexual mycelia derived from this ascus, but not nearly so many as when each was mated with the tester strain of the opposite sex. Further work would be necessary,

however, to confirm this statement, as it is an important point which should be settled on the basis of a larger number of cultures.

Four of the five mycelia obtained from ascus M' developed conidia. We have here material adaptable for a study of several interesting questions. The writer (3) has discussed the origin of the albinistic non-conidial race of *N. sitophila* with which these experiments were begun. This new race was due to a "salutation," or some sort of somatic segregation or non-distribution occurring in the Arl. 10 strain. It was proved that certain conidia develop into mycelia which are incapable in turn of producing conidia, at least to any extent. Normal bisexual mycelia of *N. tetrasperma* produce three kinds of conidia, i.e., bisexual, unisexual sex A, and unisexual sex B (9). It may be asked, what is the nature of the conidia produced by our mycelia 1, 4 and 5 derived from the bisexual hybrid ascospores in ascus M', each spore having one nucleus which does not carry the +C factors determining conidium production? Shall we have some bisexual conidia? Will some of the conidia be unisexual and if so will some of them be of one sex and some the other, as in case of normal *N. tetrasperma*? Or will some of these conidia develop into non-conidial mycelia because they were provided with only the nuclei lacking the +C factors for conidium production? Since four of the five spores in ascus M' developed conidial mycelia and as this ascus is a hybrid from a cross between non-conidial and conidial races, we assume segregation of factors for conidia. Relative to their sex, which four nuclei carry the +C factors for conidia and how can this be proved? These are a few of the questions that occur to one from time to time as he studies this interesting material. The answers to some of them appear in the results of the experiments recorded below.

*The nature of single conidia derived from mycelium 1M'.* Without taking special precautions, twenty-one single conidium cultures were obtained by the usual method. Coming from a bisexual parent mycelium it is rather strange that only one culture produced any perithecia. Culture 20 did form a few fruit bodies, but all of the other twenty have remained without ascocarps, up to date. These cultures are now about four months old. Tested thoroughly against tester strains S<sub>1</sub> and S<sub>8</sub> it was proved that all

were of sex A class. Clons 5, 11, 13 and 17 were rather weak in their reaction, but the others were very fertile with S<sub>1</sub> under the same conditions. Not one of the twenty-one mycelia failed to produce conidia, although certain ones developed only comparatively few.

*Single conidium mycelia from 4M'.* Twenty-five cultures were obtained from single conidia plated out from culture 4M'. Mycelia 4, 5, 13, 16 and 19 were dwarfs which grew so slowly that they could not be tested out for their sex. The original cultures from the other twenty conidia never produced any ascocarps. Tested out for their sex it was proved without a doubt that they were all unisexual and again all of sex A class. Furthermore, each mycelium produced conidia.

*Single conidium mycelia from 5M'.* Twenty-five cultures were obtained from single conidia from 5M' which, like nos. 1 and 4, is bisexual. Again several of these mycelia grew very slowly, but by transferring them to dextrose agar they became invigorated so that they could be studied. Every one of the twenty-five mycelia produced conidia, but no perithecia. Tested for sex, it was again proved conclusively that they were all of sex A class.

It is not at all surprising to find that all four sex A nuclei in ascus M' carried the +C factors for conidia and all sex B nuclei were non-conidial. On the other hand, it is very remarkable that of the cultures derived from single conidia developed on the bisexual mycelia, 1M', 4M' and 5M', only one out of sixty-six produced ascocarps. It was desirable in these experiments not to be too particular in selecting the conidia, being careful only to avoid transferring hyphal fragments. The results are all the more conclusive on this account as showing that only those nuclei carrying the +C factors for conidia are included in a conidium. Any conidium having non-conidial as well as conidial nuclei would be bisexual and its mycelium would produce perithecia. Only one such culture was obtained among the sixty-six studied. This one was due no doubt to the selection, as noted previously, of a proliferating hyphal fragment rather than a germinating conidium.

Three kinds of conidia are formed on the normal bisexual or hermaphroditic mycelia of *N. tetrasperma* because of the unequal

distribution of the sex A and sex B nuclei. The Arl. 10 unstable sex B race of *N. sitophila* produces two kinds of conidia, "typical" and "albinistic." The bisexual mycelia 1M', 4M' and 5M' go back for their real origin to these same parents, *N. tetrasperma* and the saltant non-conidial races of *N. sitophila*; yet we find quite a different rule followed in conidium formation. At the end of the third division in ascus M' the four nuclei containing the +C factors are all of sex A class, and only one of these is included in an ascospore. Spores 1 and 2 from ascus G, although developed in a hybrid ascus with the same parentage as ascus M', correspond to normal *N. tetrasperma* ascospores in that they contain one nucleus of each sex and each nucleus carries the +C factors. In forming conidia their mycelia behave like *N. tetrasperma* in developing three kinds of conidia. The other four nuclei in ascus G were non-conidial.

These results help to explain the peculiar behavior of mycelia obtained from other asci, notably asci J and L, where all of the mono-conidial mycelia obtained by plating conidia were unisexual and sex B. The delayed and erratic production of ascocarps in the original cultures derived from their ascospores was no doubt due to certain incompatibilities or types of self-sterility. The continued sterility of most of the sub-cultures, however, might be due to transferring only conidia, which would mean inclusion of only sex B elements. Such an explanation is a very doubtful one, as sub-cultures were usually made by transferring bits of agar containing mycelium rather than merely conidia. It seems more likely that in the growth processes the nuclei of one sex divide more rapidly than do those of the other sex, so that in certain areas of growth the hyphae contain only nuclei of one sex. This might be only a temporary condition, but if transfers were made from such a place a unisexual strain would be started. Whatever may be the true explanation of the irregularities in sexual reproduction and conidium formation encountered in the culture experiments described in this report, it is clear that the rôle of the cytoplasm and nuclei respectively in these processes is a subject for a more extended study.

### HERMAPHRODITIC NON-CONIDIAL 4-SPORED HYBRIDS

One of the aims in making the interspecific crosses was to obtain a bisexual or hermaphroditic strain with a fluffy albinistic non-conidial aerial growth like strain 56.2, which was the 8-spored *sitophila* parent. It was also desired that the hybrid should have 4-spored ascospores like *N. tetrasperma*. It has been shown that strains no. 4, ascus C, and nos. 3 and 4, ascus G, were bisexual, non-conidial, and the ascospores in their perithecia were fairly regularly 4-spored. Their aerial mycelia, however, were rather scanty. It was not at all difficult to keep the two characters, albinistic and non-conidial, together in the back-cross hybrids between no. 56.2, *sitophila*, and conidial *crassa*. When, however, *crassa* with its fluffy aerial growth and abundance of conidia was replaced with strain 507 with its scanty aerial growth and few conidia, difficulties arose. The chances were very much against getting together in the same ascospore two nuclei of opposite sex, each lacking +C factors for conidia and each carrying factors for the luxuriant aerial mycelium. Strain S<sub>6</sub> was used as the 4-spored back-cross parent instead of no. 507. It likewise produces very little aerial growth and not very many conidia. S<sub>6</sub>, being straight *tetrasperma*, may be presumed on this account to be more regularly 4-spored than the segregate no. 507. In the endeavor to learn something of the nature of the "weakly" bisexual strains and the way segregations of the factors for sex and for conidia were occurring in the 4-spored hybrid ascospores, the fluffy albinistic feature was not made the main basis for the selections. A segregate with the desired combination of characters listed above could have been obtained more readily in another way. From the cultures which gave the most 4-spored ascospores in each case, much larger numbers of the small unisexual ascospores should have been grown, so that several pairs of mycelia of opposite sex, each one with the luxuriant albinistic growth, were available for matings from which to select for 4-sporedness. Something of this sort was done incidentally when clons 22 and 34 were selected from among thirty-four back-cross haplotypes of a second mating, {(507 × 56.2)<sub>29</sub> × S<sub>6</sub>}<sub>8</sub> × S<sub>6</sub>. They were of opposite sex, somewhat albinistic, and non-conidial. The basis for their selection aside from being non-conidial was that when grown together a great abundance

of ascocarps was obtained, a doubtful procedure when this was not the particular character desired.

The asci from this mating were mostly 4-spored; yet there was enough irregularity as indicated by the frequency with which one found asci with giant spores as well as asci with five or six spores to enable one to readily distinguish these ascocarps from those produced by typical *N. tetrasperma*. By continued selfing one could obtain bisexual non-conidial strains which would produce 4-spored asci very regularly.

Four 4-spored asci from the mating 22 × 34 were analyzed. Some of the characteristics of the mycelia in each case are given to indicate that the fertility of the parent combination is not always passed on to the offspring, and that the fluffy albinistic type of growth has not been perpetuated through the various back-crossings.

Ascus A. 1. Non-conidial, bisexual, slowly fertile at first, then prolific.  
2. Non-conidial, bisexual, very fertile.  
3. Non-conidial, bisexual, very fertile although ascocarps appeared slowly at first.  
4. Non-conidial, bisexual, abundance of perithecia.

All of these four mycelia produced only a scanty aërial growth; most of the asci were good 4-spored types.

Ascus B. 1. Non-conidial, bisexual, very fertile.  
2. Non-conidial, bisexual. Thought to be sterile at first but a few perithecia finally matured.  
3. Spore did not germinate.  
4. Non-conidial, bisexual, very fertile.

Ascus C. 1. Spore did not germinate.  
2. Non-conidial, bisexual, very fertile.  
3. Non-conidial, bisexual, slowly fertile. Asci fairly regularly 4-spored.  
4. Non-conidial. No perithecia have developed in culture.

Ascus D. 1. Non-conidial, very weakly bisexual.  
2. The same as no. 1.  
3. Non-conidial, strongly bisexual.

Clon 2A, which is strongly bisexual, and 3C, which is rather

weakly bisexual, were among the so-called homothallic strains which were later hybridized directly with certain unisexual strains.

Twelve ascospores of normal size taken at random from the spore print on tube 22 X 34 gave non-conidial bisexual mycelia. All produced an abundance of perithecia except no. 3, which developed only a few after many days. The asci were mostly 4-spored. Clon 8 in this series was the only one which had anything resembling the fluffy albinistic type of aerial growth.

#### CROSSING HERMAPHRODITIC WITH HETEROOTHALLIC RACES

The discovery that certain species of *Mucor* were heterothallic first led Blakeslee to attempt to obtain hybrids. The possibility of hybridizing species of ascomycetes was not seriously considered until very recently. No doubt it was deemed futile to try to cross species unless mycelia of opposite sex were developed. Although Thaxter had previously described several species of the Laboulbeniales as "dioecious," it was not until the life history of *Ascobolus magnificus* was studied (4) that any ascomycete was proved culturally to be heterothallic. Strangely enough the first hybrid ascomycetes produced were between species rather than between races of the same species. Crossing species of yeasts such as *Schizosaccharomyces octosporus*, where fertilization is assured by the fusion of two free bud cells, would not be a question of heterothallism versus homothallism. It would all depend on the efficacy of cross fertilization as compared with close inbreeding through self fertilization. The same would be true for forms like *Taphrina epiphylla*, which is known to be heterothallic (13).

Every nucleus in the mycelium of *Pyronema*, up to the time of the differentiation of the sex organs, carries the potentialities for complete sexual reproduction. The chances of getting such a species to hybridize would seem on first thought to be remote. It was only because *Neurospora tetrasperma* occasionally develops a small unisexual ascospore that the crossing of this species with heterothallic *N. sitophila* seemed worth trying. One may argue that *Pyronema* and *Neurospora tetrasperma* are not comparable in their homothallism because in the latter species there are two kinds of nuclei as to their sex. But so there are in *Pyro-*

*nema* after the differentiation of the two branching systems giving rise to oögonia and antheridia respectively.

Several examples were given in previous pages where a monosporous mycelium, which appeared for a long time as tested to be unisexual, finally gave rise to a few ascocarps. The ascospore producing such a mycelium must have originally contained one nucleus of each sex. Many examples are recorded in the literature on culturing ascomycetes of the effect of bacterial or other fungous contaminants accidentally or intentionally introduced into the culture. *Ascobolus Winteri* is homothallic. Yet if one inoculates a plate culture on opposite sides with transplants from the same monosporous culture two rows of ascocarps are formed along the line where the two mycelia meet (5). With such examples as these in mind when it was found that the weakly bisexual strains referred to above fruited abundantly with the one or the other tester strains S<sub>1</sub> and S<sub>6</sub>, the question arose as to whether the tester strain may have been acting merely as a nutritive contaminant, not taking any part whatever in fertilization. On the other hand if the progeny from the ascocarps showed characters, certain ones of which were present only in the tester strain, this would prove a preference for cross fertilization to selfing, thus suggesting a way of hybridizing homothallic strains. Experiments planned to prove that this can be done will be described presently.

The origin of the strains mentioned previously and in connection with the matings to be described in succeeding paragraphs can be seen at a glance in the following tabulation.

- S<sub>6</sub>. A unisexual sex A haplont of *tetrasperma*.
- S<sub>1</sub>. A unisexual sex B haplont of *tetrasperma*.
- 209.  $\{(sitophila \times tetrasperma)_1 \times S_1\}_9 =$  sex A haplont.
- 507.  $(209 \times S_1)_7 =$  sex A haplont.
- 56.2. Non-conidial sex B haplont of *sitophila*.
- Asci C', G, L and M'.  $\{(507 \times 56.2)_{29} \times S_6\}_8 \times S_6.$
- 1C'. Bisexual mycelium derived from spore no. 1 from ascus C'. The nuclei of only one sex carry +C factors for conidia.
- 1G, 2G. Bisexual mycelia derived from spores 1 and 2 from ascus G. All nuclei of both sexes carry the +C factors for conidia.
- 3G, 4G. Bisexual non-conidial mycelia from spores 3 and 4, ascus G.
- 5M'. Bisexual mycelium from spore no. 5, ascus M'. Only the sex A nuclei carry +C factors for conidia.
- 22.  $\{[(507 \times 56.2)_{29} \times S_6]_8 \times S_6\}_{22} =$  Non-conidial sex A haplont.

34. Sex B haplont. Same origin as no. 22; also non-conidial. Ascii (4-spored) A, B, By, C. Ascii from the mating 22  $\times$  34. 1A, 2A, 2B, 2By, 3C, etc. Bisexual non-conidial mycelia from spores from ascii A, B, By and C respectively.

The distribution of perithecia in a plate culture of *N. tetrasperma* inoculated on one side with strain S<sub>1</sub> and on the opposite side with strain S<sub>6</sub> is characteristic. The first fruit bodies begin to form where mycelium S<sub>6</sub> meets S<sub>1</sub>. Others appear one by one back along the lines of growth of S<sub>6</sub> hyphae radiating from the point of inoculation. Ascocarps do not form on the area occupied by S<sub>1</sub> mycelium (PLATE 7, FIG. 17). This does not prove however that strain S<sub>6</sub> is female. When a culture of *Ascobolus magnificus* (4) is prepared in a similar manner one readily sees where the two mycelia come together, stop growing for a short time, then grow right on over so that there is a complete intermingling of the mycelia of both sexes. Finally ascocarps are found scattered here and there all over the culture. Should there be any such intermingling of hyphal elements in cultures where strains S<sub>1</sub> and S<sub>6</sub> of *N. tetrasperma* are grown from opposite sides it is certainly not readily detected. Inoculate a plate culture with strain S<sub>1</sub> alone, and, after the new mycelium has covered the plate, transfer to this plate a small block of agar taken from a similar culture containing only S<sub>6</sub>. Ascocarps develop principally, if not exclusively, on the little block of S<sub>6</sub> agar. Reverse the process and transfer a little block containing S<sub>1</sub> mycelium to a plate culture of strain S<sub>6</sub> and no ascocarps form on the little block, but they do develop right beneath it and then spread out in the S<sub>6</sub> area. The area finally containing fruit bodies is usually not large however. These few experiments are presented to show certain peculiarities or cultural characteristics which have been found helpful in explaining the results obtained in other experiments.

The picture formed when a plate culture is inoculated on opposite sides with normal bisexual mycelium of *N. tetrasperma* in which every nucleus carries the +C factors for conidia is shown in PLATE 5, FIG. 1. Ascocarps first appear at the points of inoculation and then spread out in radiating lines. Instead of forming a dense line of perithecia where the two mycelia meet we have a zone comparatively sterile. This zone gradually

narrows as the culture ages. Yet one sees a certain antagonism which inhibits sexual reproduction although the two mycelia leave no area unoccupied.

A similar picture (PLATE 5, FIG. 2) is developed when the culture contains two plantings of 5M' in which only sex A nuclei carry the +C factors for conidia. Although 5M' is a hybrid segregate many of its ascii are 4-spored. Compare the distribution of ascocarps in the two cultures just described with what one finds when either *N. tetrasperma* or 5M' is grown opposite S<sub>1</sub>, which is sex B and conidial (PLATE 5, FIGS. 4, 6). So far as one can see S<sub>1</sub> takes no part here in sexual reproduction, serving only to block the advance of the bisexual mycelia which are producing ascocarps right up to the line of meeting. When however strain S<sub>6</sub>, which is sex A and less strongly conidial, is grown opposite either *N. tetrasperma* or 5M', a somewhat different reaction occurs (PLATE 5, FIGS. 3, 5). Here quite a few fruit bodies are formed on the S<sub>6</sub> mycelium, many of them being distributed down along the lines of hyphal growth. Consider first the culture containing *tetrasperma* and S<sub>6</sub>. Does S<sub>6</sub> take an active part in the sexual process, or are the ascocarps wholly the product of the bisexual *tetrasperma* mycelium which has now grown down over the S<sub>6</sub>, perhaps because S<sub>6</sub> is less antagonistic to it than is S<sub>1</sub>? It would be difficult to prove which is the correct answer because of the maze of fine hyphal branches growing in all directions in cultures of this age. Furthermore the ascocarps would be typical *tetrasperma* in either case because S<sub>6</sub> is merely unisexual sex A *tetrasperma*.

The solution of the problem would be scarcely less difficult in case of the culture where 5M' is grown with S<sub>6</sub> (PLATE 5, FIG. 5) because the nuclei in 5M' which carry the +C factors for conidia are of the same sex A as are those of mycelium S<sub>6</sub>. It has been stated previously that S<sub>6</sub> was the back-cross parent in the origin of 5M', so that it would be a long and difficult undertaking to prove by analysis whether the S<sub>6</sub> here was again the back-cross parent in the production of the ascocarps now formed on the area occupied by it in this culture.

When the non-conidial haplonts nos. 22 and 34 are mated in culture the ascii formed are mostly 4-spored. Here also the 5- and

6-spored ascospores are numerous enough to enable one to distinguish the perithecia from those formed by *tetrasperma*. Plate cultures were prepared by inoculating one side with *tetrasperma* and the opposite side with either no. 22 or no. 34. Perithecia formed as usual on the area occupied by the *tetrasperma* mycelium, being most abundant around the point of inoculation, thinning out abruptly as this mycelium approached the one from the opposite side. In the meantime, many ascocarps had developed in a narrow zone along the line in each case where the two mycelia met (PLATE 6, FIGS. 7, 8). No. 34 is sex B; yet if we replace it with S<sub>1</sub>, no such line of perithecia is formed, as previously noted (PLATE 5, FIG. 4). There can be no question that the hermaphroditic *tetrasperma* has hybridized directly with both no. 22 and no. 34 in the cultures figured. The fruit bodies are larger than are those formed on the *tetrasperma* area. There is more than enough irregularity in the number of spores in the ascospores to prove hybridity.

Reverse the process, and grow the hermaphroditic race 2A on one side and either S<sub>6</sub> or S<sub>1</sub> on the opposite side, and we find exactly the same picture formed (PLATE 6, FIGS. 9, 10). The reason is clear. S<sub>6</sub> and S<sub>1</sub> are *tetrasperma* with the sexes separated in two different mycelia, and 2A is simply the product of a mating or a union between 22 and 34.

Clon 3C is weakly bisexual. It has the same origin, however, as 2A. When 3C is grown opposite either S<sub>6</sub> or S<sub>1</sub>, ascocarps form first along the line where the 3C meets the S<sub>6</sub> or the S<sub>1</sub>, as the case may be. Spores are discharged from these ascocarps several days before fruit bodies on the 3C area are fully developed (PLATE 6, FIGS. 11, 12). The hybrid nature of the ascocarps along the lines of meeting of 3C and S<sub>1</sub> has been proved beyond question. They are fully twice as large as are those of 3C. Both series of perithecia have irregularity in the number of spores in their ascospores, so this feature could not be used as proof of the point in question. If strain S<sub>1</sub> has contributed nuclei which have entered into the fusion nuclei in the ascospores, some of the ascospores should produce mycelia with monilioid conidia. From among the ascocarps at the center of the line, two or three which had formed wholly beneath the surface of the agar were selected.

This was done not only to avoid the possibility of carrying over ascospores which might have been shot over from the 3C area, but also to lessen the chances of contamination by conidia from the S<sub>1</sub> area, which was the important thing.

Some of spores of the first sowing germinated without heating. Sixteen, judged by their small size to be unisexual, were transferred, and the remainder were given a severe heat treatment to kill the conidia present, which were now germinating rapidly. Many more ascospores were stimulated to grow. Forty-three were selected at random without regard to their size, and four isolated from one ascus were also included. The sixteen spores which had germinated without heating all gave rise to non-conidial mycelia. Only one was bisexual; the others were all of the same sex, A. All of the forty-three mycelia derived from the heated spores produced conidia in varying amounts. Thirty-three were bisexual and ten were unisexual, of which five were sex A and five were sex B. The production of conidia by so many of these single ascospore mycelia proves most conclusively that haplont S<sub>1</sub> had actually hybridized with the bisexual, totipotent race 3C. Mr. Carl C. Lindegren, who is especially interested in determining why some spores germinate without heating while others require severe heat treatment, has conducted some further experiments, analyzing individual asci from this same cross. He is including these results in a paper shortly to be sent to press. On the main question raised above, namely, the parentage of the ascocarps developed along the line where mycelia 3C and S<sub>1</sub> meet, his results furnish all the supporting evidence that could be desired for the conclusion that haplont S<sub>1</sub> is the sex B parent. Mycelium 3C contains nuclei of both sexes which unite in ascocarps arising from self fertilization. Given an opportunity where the two mycelia meet, cross fertilization with S<sub>1</sub> occurs. Haplont S<sub>1</sub> contributes the sex B nuclei and mycelium 3C only sex A nuclei.

It will be seen from the picture presented (PLATE 6, FIG. 11) that, in the plate culture in which S<sub>8</sub> was grown opposite 3C, ascocarps are formed along a rather broad zone where the two mycelia meet in much the same manner as in a culture of 3C with S<sub>1</sub>. Other cultures of 3C + S<sub>8</sub> show a double line of fruit bodies. One can see the double line more distinctly in the reverse view

of the plate culture (PLATE 7, FIG. 13), which shows that many ascocarps here developed wholly beneath the surface of the agar. The fruit bodies on the  $S_6$  side were very likely hybrids between 3C and  $S_6$  and therefore some of the ascospores should give rise to mycelia with monilioid conidia like  $S_6$ . On the other hand, it might be that the ascocarps along the line on the 3C side were wholly the product of strain 3C alone, forming fruit bodies along the line where further growth was checked, or where the nutrient was being exhausted. In this case all of the ascospores should give rise to non-conidial mycelia. With the view of learning the meaning of this double row of fruit bodies, cultures were obtained from their ascospores. Two ascocarps which had developed beneath the surface of the medium at the point "X" on the  $S_6$  side and two others "Y," from the 3C side, were selected for the test. The crushed mounts did not show any particular differences. In both sets the asci were mostly 4-spored, with some 5-spored asci characteristic of these back-cross hybrids mixed in with them. The cultures derived from spores taken from the "X" perithecia formed on the  $S_6$  side of the zone will be noted first.

From among the spores which germinated without heating twenty-one were chosen at random without regard to their size. Twenty of their mycelia produced conidia, and only one was non-conidial. This was just the reverse of the case in the mating 3C  $\times S_1$  where the sixteen spores which germinated without heating were all non-conidial. One of the mycelia produced a few perithecia. The others were all tested against  $S_1$  and  $S_6$ . Eleven finally developed perithecia when grown with  $S_1$ , reacting as sex A. The other nine did not produce any fruit bodies when grown with either  $S_1$  or  $S_6$  in two separate tests, illustrating again rather strong incompatibilities.

After removing some of the spores which germinated without heat, the plate was given the heat treatment. Practically all of the remaining spores germinated. Twenty-seven of their mycelia produced conidia and three did not. Twelve mycelia developed many ascocarps and were therefore bisexual. The other fifteen were all tested against both  $S_1$  and  $S_6$ . Thirteen produced no ascocarps when grown with either one of these two

tester strains. It has been noted previously that certain mycelia of such hybrids produce perithecia only after many weeks in culture. This proves that they were really bisexual. No doubt something of this sort is operating here, because a larger proportion of the mycelia from ascocarps chosen more or less at random should have developed ascocarps readily. Much of the self- and cross-sterility exhibited in this series was very likely due to incompatibilities which should be made evident when other tester strains are employed. These tests were not carried further because the main point in question, namely, the nature of ascocarps along the line of the  $S_6$  side of the culture, was settled. The hermaphroditic mycelium 3C does not produce monilioid conidia, and  $S_6$  alone does not develop ascocarps. The conidial mycelia obtained from these ascospores are proof that 3C contributed the sex B nuclei and  $S_6$  furnished the sex A nuclei which combined in the hybrid ascii. It so happened that most of the ascospores selected at random gave conidial mycelia just as would be expected. No doubt an analysis of the spores from 5-spored ascii would show that about half of the small unisexual spores would give rise to non-conidial mycelia.

Spores from the ascocarps "Y" from the 3C side of the culture 3C +  $S_6$  which made the double line of ascocarps (PLATE 7, FIG. 13) were sowed in the usual way. Nineteen spores which germinated without heating were selected at random. Three of their mycelia were bisexual and the other sixteen were unisexual, sex A. Ten spores which required heat to induce germination were bisexual and one was of the type with weak sexuality. It reacted with neither  $S_1$  nor  $S_6$ , the only strains it was tested against. The fact that all of the bisexual mycelia were non-conidial is the best possible proof that the ascocarps along the line on the 3C side were the products of this strain alone. The meeting of 3C and  $S_6$  brought about a condition favoring production of ascocarps by the bisexual strain. The  $S_6$  mycelium merely acted as an obstruction to further growth of the 3C mycelium. A colony of bacteria, some other fungus, or the side of the petri dish (PLATE 7, FIG. 14) might serve as well. With an equal opportunity for either self-fertilization or cross-fertilization, which method would prevail would depend on what strains are grown together.

Certain writers have interpreted the production of ascocarps along the line of meeting of two mycelia as proof of heterothallism. An analysis of the progeny from individual asci would show whether this were true or not.

The evidence presented certainly justifies the conclusion that certain hermaphroditic strains will hybridize directly with certain unisexual strains. The formation of large hybrid ascocarps along the line of meeting suggests the working out of a preference for cross-fertilization over selfing. Such hybridizing goes on equally well with either sex,  $S_6$  and  $S_1$ , on the one hand, or no. 22 and no. 34 on the other.

#### CROSSING TWO HERMAPHRODITIC RACES

We have seen that neither of the hermaphroditic strains *tetrasperma* and 5M' crosses with  $S_1$  in a plate culture, while 1C', 3C and 2A do so readily. With  $S_6$ , 2A acts much like 3C. Some cultures show two distinct lines of fruit bodies along the zone where the two mycelia meet. No doubt the ascocarps along the line on the 2A side are the product of 2A alone, while those on the  $S_6$  side are hybrids between the bisexual totipotent 2A and the haplont  $S_6$ .

Several different bisexual non-conidial strains such as 3C, 2B, 2By, 3G, 4G and "42," whose asci are mostly 4-spored, have been developed. These strains have been grown in plate cultures opposite bisexual conidial strains such as straight *tetrasperma*, 1G and 2G, where all of the nuclei carry the +C factors for conidia. Each strain in every culture produced its own ascocarps and these ascocarps were variously distributed. In some cases the fruit bodies scattered irregularly along the line of meeting were clearly due to checking of growth. Distinct lines of ascocarps were formed in cultures of several different matings in such a way as to leave little doubt that cross-fertilization and hybridization had occurred between the two mycelia, each totipotent in itself (PLATE 7, FIG. 18).

*N. tetrasperma* formed such a line with clon 1C' in plate cultures. Some of the fruit bodies were clearly pure *tetrasperma*. Their asci were very regularly 4-spored. Others showed odd-shaped over-sized ascospores and not a few 5-spored asci. It

would not always be easy to prove them hybrids, *tetrasperma*  $\times$  1C', as distinct from ascocarps, the product of 1C' itself. One of the two original nuclei included in spore 1C' carried the +C factors for conidia. If, for example, this nucleus was of sex A, and clon 1C' contributed the sex A nuclei and *tetrasperma* the sex B nuclei, then, besides numerous 4-spored asci, one would find quite a few with five or six spores. Furthermore, every ascospore, large or small, would give rise to a conidial mycelium. This would also be true for pure *tetrasperma*, but the percentage of 5-spored asci would be much smaller. Should clon 1C' contribute its non-conidial sex B nuclei in a mating, and *tetrasperma* the sex A nuclei, then the resulting ascocarps could scarcely be distinguished by any test from those formed by 1C' alone.

Another mating of two hermaphroditic clons the hybrid nature of whose ascocarps could be readily distinguished from those formed by either of the bisexual parents through self-fertilization would be such that in one mycelium only the sex A nuclei carry the +C factors for conidia while in the other mycelium the +C factors are carried only by the sex B nuclei or vice versa. The sex A nuclei in clon 5M' are conidial, the sex B nuclei being non-conidial. The sex B nuclei in clon 3L are conidial and the sex A nuclei are non-conidial. Hybrid ascocarps between these two races would give ascospores which would all be either conidial or all non-conidial, depending on which parent furnished the sex A nuclei. No ascii would have both kinds of spores. Asci from perithecia formed by either parent through selfing would always show segregation of the factors for conidia, so that some of the small spores would be conidial and some would be non-conidial.

Most of the matings between hermaphroditic strains were made in plate cultures on 4 per cent corn-meal agar. Such hard agar stimulates aerial hyphal growth, but often retards fruiting. The results of the preliminary experiments represent only one or two cultures of each pair grown together. Further work culturing from spores produced by the ascocarps formed along the line of meeting may show that what are now taken to be hybrids are merely products of self-fertilization by the one or the other strain. The following pairings of bisexual clons produced a line

of fruit bodies where the mycelia met, in addition to those produced by each one (*N. tetrasperma* is abbreviated "Tet"): Tet  $\times$  3C; Tet  $\times$  2B; Tet  $\times$  2By; Tet  $\times$  3C; Tet  $\times$  3G; Tet  $\times$  4G; 1G  $\times$  42; 2G  $\times$  1A.

No line of ascocarps was formed where the following bisexual mycelia met in plate culture: Tet  $\times$  5M'; Tet  $\times$  Tet; 5M'  $\times$  5M'; 2G  $\times$  2B; 2A  $\times$  2C; 2G  $\times$  3C; 2G  $\times$  4G; 1C'  $\times$  2By; 2G  $\times$  42; 3C  $\times$  3C.

Practically the only place where crushed mounts would serve to indicate hybridity would be where pure *tetrasperma* was grown opposite a strain which formed asci with noticeable irregularities as to the number of spores in the asci and spore shapes and sizes. For example, three different types of ascocarps are readily distinguished in culture Tet  $\times$  2B. Crushed mounts of fruit bodies from the *tetrasperma* area show great uniformity and abundance of 4-spored asci. Those from the 2B area show irregularity. Ascocarps taken from the line of meeting where the two mycelia could hybridize show some irregularities such as might be expected of intermediates. Culturing mycelia obtained from the ascospores selected at random, while not altogether dependable, usually serves to settle the question. The simplest and the best way, in case of the cross Tet  $\times$  2B, would be to grow each spore from just one 5-spored ascus. If the five spores all produce conidial mycelia, the ascocarp originated from *tetrasperma* alone by selfing. If all five spores produce non-conidial mycelia, the ascocarp arose on mycelium 2B without crossing with *tetrasperma*.

This line of reasoning is based, of course, on the assumption that if two hermaphroditic races cross, one race contributes only sex A nuclei while the other race furnishes only sex B nuclei. Should, however, the perithecial primordia include one or more pairs of nuclei of both sexes from each race, complications would arise. With a nuclear fusion in the ascogenous cell, followed by a second fusion in the young ascus, various types of asci would be developed from which different kinds of haplont progeny would be segregated out. It is not inconceivable that cross-fertilization might be of such a nature that two nuclei of opposite sex from one race and only one nucleus from the other race enter the primordia. Some of the F<sub>1</sub> asci might contain the inheritance of

one parent only, and the other asci might be hybrids. At this writing the work has not reached a point where it is desirable to warrant positive conclusions as to the nature of the fruit bodies developed along the line of meeting such as shown in PLATE 7, FIG. 18.

The use of the term hybrid in connection with the experiments reported above has perhaps been a rather loose one. A peritheciun has been referred to as a hybrid when it is meant, of course, that the asci within it are hybrid. The framework of an ascocarp is very likely purely gametophytic. It may be a mixture of such hyphal elements from both parents, or it may be formed from the hyphae of the one or the other parent mycelium without altering the nature of the asci within.

In their report on a re-investigation of *Sphaerotheca*, *Polystigma* and *Pyronema*, the Moreaus (12) have included the results of their cytological study of the three species of *Neurospora*. They state that the ascogenous cell contains at first several nuclei, all but one of which degenerate. The ascogenous hyphae then grow out from this uninucleate cell. The cells of the ascogenous hyphae are also uninucleate. The crosier or ascus hook arises from a stalk-cell which has only one nucleus. There is no antheridium or other structure which can be seen to function as such. The writer is not ready as yet to say whether this account of nuclear behavior in the origin of the ascogenous elements of *Neurospora* is correct or not. Leaving the subject of sex aside for the time being, there is still to be explained, on the basis of the facts presented by these authors, the very definite Mendelian inheritance of the albinistic non-conidial characters in matings such as have been described by the writer previously (3) and in the present paper.

If the crosier arises from a cell containing only one nucleus, as stated by the Moreaus, that nucleus must in some way have become heterozygous for the characters being considered. Was this through a previous nuclear fusion in the ascogenous cell similar to that described by Harper, Gwynne-Vaughan and others? If so, how did nuclei from two different mycelia get into that cell? If no antheridium is present, was it originally through an anastomosing of vegetative hyphae? The picture presented

(PLATE 7, FIG. 17) when clon  $S_1$  is grown opposite clon  $S_8$  is highly interesting. Such a striking pattern must have some significance.  $S_8$  contains only sex A nuclei. Sex B nuclei, which can be had only from  $S_1$ , in some way have been brought to every point now showing a peritheciun. Perhaps it will be through genetical studies of cultures like those shown in plates 5 to 7, supplemented by cytological work, that the questions regarding nuclear activities in sexual reproduction, which have been in heated dispute for the last thirty or forty years, will become better understood. *Neurospora sitophila* is world wide in its distribution. *N. crassa* can be found in tropical countries and wherever sugar cane is grown. *N. tetrasperma* can be obtained at least from a number of culture laboratories. All three species are readily cultured, and with some patience can be made to hybridize and produce fertile offspring. They are admirably suited, therefore, for further genetical studies.

#### SUMMARY

Further experiments in crossing typical conidial races of the *Monilia* bread mold, *Neurospora sitophila*, with albinistic non-conidial races of the species indicate that asci from such a mating are heterozygous for conidia, and that segregations of the factors for sex and the factors for conidia occur independently. Both sets may segregate in the first nuclear division in the ascus, both sets in the second division, or one set in the first and one set in the second. The eight spores may thus alternate in the ascus: two and two; four and four; or two, four, two, as to their sex or the factors for conidia which they contain.

The albinistic non-conidial race of *N. sitophila* was crossed with typical conidial *N. crassa* and fertile hybrids were obtained. The  $F_1$  asci usually mature only two or three spores. Of the forty-four haplont clons studied, only six were strictly non-conidial. Five were albinistic intermediates producing a few conidia abnormally. Thirty-three were conidial, resembling the *crassa* parent in coloration. The albinistic non-conidial  $f_1$  strains were back-crossed with the *crassa* parent. The hybrids obtained were very fertile, with little abortion of the ascospores. The asci and spores resemble more those of the *crassa* parent. The

few asci analyzed showed that in some cases the segregation of the factors for conidia was completed either in the first or the second nuclear division, so that the spores are disposed two and two; four and four; or two, four, two; as was proved to be the case for intraspecific crosses in *N. sitophila*. Some evidence was obtained indicating that in these back-cross asci segregation of the factors for conidia is occasionally not perfect, resulting in intermediate types with albinistic aerial growth, but with a few conidia rather abnormally formed.

Hybrids were obtained by crossing the albinistic non-conidial race of *N. sitophila*, which has 8-spored asci, with a weakly conidial, "scanty growth," 4-spored segregate 507, resembling *N. tetrasperma* as to its asci. The  $F_1$  asci were 8-spored, but with much spore abortion after delimitation. Over 60 per cent of the  $f_1$  haplonts grown from ascospores chosen at random were non-conidial. Some of these were fluffy albinistic types; others produced only a scanty aerial growth. Some haplonts produced highly colored conidia in far greater abundance than does the conidial 4-spored parent. When the non-conidial  $f_1$  haplonts were crossed with  $S_6$ , pure *N. tetrasperma*, the asci developed in the fruit bodies from this mating contained variable numbers of spores, mostly from three to six. Mycelia obtained by growing the small unisexual spores showed still greater variation in the color and abundance of conidia produced. Again over 60 per cent of such haplonts did not produce conidia. Non-conidial segregate haplonts were again crossed with *N. tetrasperma* with the result that now many asci with four spores were produced. Spores from several asci were isolated in order and germinated. A number of the hermaphroditic mycelia obtained showed either weak sexuality or incompatibilities, some even approaching neutrals; otherwise segregations of the factors for sex and for conidia appeared to be Mendelian. Both nuclei in a bisexual spore from these hybrid asci may carry +C factors for conidia; one of the nuclei may be conidial and the other not; or both nuclei may be non-conidial, depending on the way the segregations of factors for sex and for conidia take place and the redistribution of the nuclei before the spores are cut out. When both nuclei carry the +C factors, the mycelium from that spore will develop

three kinds of conidia as regards their sex. If only one of the two nuclei in a bisexual spore carries the +C factors, then only one kind of conidia as to sex will be cut off.

Hermaphroditic, totipotent races were crossed with strictly unisexual races, and fertile hybrids were obtained as the result of a preference for cross-fertilization over selfing, the ascocarps forming along the line in each case where the two mycelia met in plate cultures. It was proved also that lines of ascocarps may be formed across a plate culture in which two such mycelia are grown, without any act of cross-fertilization entering into the phenomenon.

Some hermaphroditic races, such as pure *tetrasperma* and 5M', which produce ascocarps freely on their own mycelia, when grown opposite each other in plate culture do not form lines of fruit bodies where the two mycelia meet. When certain other hermaphroditic strains, however, are grown opposite these races, such lines of ascocarps are formed where the two mycelia meet, and these fruit bodies are in addition to the ones produced by each mycelium over the area occupied by itself alone.

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#### EXPLANATION OF PLATES<sup>1</sup>

##### PLATE 1

*Neurospora sitophila*. Above. The albinistic non-conidial race of the common *Monilia* bread mold grown on white bread under a bell jar. The origin of this race is referred to in the text.

Below. The typical conidial race of the *Monilia* bread mold grown under the same conditions as the preceding. Note the masses of orange colored conidia which have fallen off. If the two races are mated in culture half of the progeny will be albinistic and half orange-colored, as shown in plate 2.

##### PLATE 2

*Neurospora sitophila*. Eight cultures showing the characteristics of the mycelia derived from the eight ascospores from an ascus produced as the result of crossing two races such as are shown in plate 1. Cultures 1 to 4 are albinistic and non-conidial; nos. 5 to 8 are typical conidial. Segregation of the factors for conidia occurred in the first nuclear division in the ascus. The sex of each mycelium is indicated by a colored circle on the tube, a green circle for sex A and a red circle for sex B. Segregation of the sex factors occurred in the second nuclear division.

##### PLATE 3

*Neurospora sitophila*. Above. Cultures representing the mycelia from the spores from a single ascus developed as a result of crossing two albinistic non-conidial strains like nos. 2 and 4 shown in plate 2, indicating that these races breed true for this albinistic non-conidial character. Sex factors segregated in second division.

Below. Cultures from the eight spores from a single ascus developed as a result of mating two typical conidial strains like strains nos. 6 and 8 shown in plate 2. These mycelia will always produce monilioid conidia in culture, and represent the common *Monilia sitophila* bread mold of older authors. Segregation of sex factors in the first division.

##### PLATE 4

Above. Nine cultures from ascospores of the mating (56.2 × 507)<sub>47</sub> × S<sub>1</sub> chosen at random. Cultures 3, 4, 8 and 9 produced conidia; the others were non-conidial.

<sup>1</sup> Plates 1 to 4 are reproduced from photographs hand colored by Miss Fleda Griffith to whom the writer is much indebted.

Below. Ten cultures from spores of the mating  $(56.2 \times 507)_{29} \times S_6$ . Cultures 2, 6 and 11 produced conidia; all of the others were non-conidial. Such high coloration and profusion of conidia were not shown by either of the original parents.

## PLATE 5

Fig. 1. Distribution of ascocarps in cultures inoculated on opposite sides with pure *tetrasperma*. Very few ascocarps develop along the line of meeting.

Fig. 2. Clon 5M' is a hybrid segregate with many 4-spored asci. When planted on opposite sides of the culture the distribution of ascocarps is much the same as in the preceding. Compare with figure 16 in plate 7.

Fig. 3. Normal hermaphroditic *tetrasperma* grown opposite clon  $S_6$ , which is unisexual sex A *tetrasperma*. The ascocarps on the  $S_6$  area are the products of cross-fertilization, the sex A nuclei being contributed by strain  $S_6$  and the sex B nuclei by the bisexual *tetrasperma*.

Fig. 4. Same as the preceding, except that unisexual sex B clon  $S_1$  replaces the strain  $S_6$ . Here no ascocarps are formed on the  $S_1$  area. (See plate 7, fig. 17, for a picture of  $S_6 \times S_1$  in culture.)

Fig. 5. Clon 5M', when grown opposite  $S_6$ , produces many perithecia by self-fertilization, and also other perithecia by cross-fertilization with strain  $S_6$ .

Fig. 6. No ascocarps are formed on the area occupied by the mycelium  $S_1$  in a culture with 5M'. Ascocarps are produced only by self-fertilization.

## PLATE 6

Fig. 7. Hermaphroditic conidial *tetrasperma* grown opposite clon 22, a non-conidial sex A race. Above are ascocarps of *tetrasperma* by self-fertilization. Hybrid ascocarps are formed along the line where the two mycelia meet. Four of the nuclei in each ascus will be non-conidial, like those in no. 22, and four will carry the +C factors for conidia contributed by *tetrasperma* sex B nuclei.

Fig. 8. Same as preceding except that no. 34, sex B race, replaces no. 22. In this case *tetrasperma* sex A nuclei enter the cross and carry conidial factors. (See plate 7, fig. 15, for a culture of the pair 22  $\times$  34.)

Fig. 9. Clon 2A, which is one of the progeny of 22  $\times$  34, produces ascocarps abundantly by self-fertilization and also a line of hybrids with  $S_6$ .

Fig. 10. Clon 2A  $\times$   $S_1$ . A line of hybrid ascocarps between 2A and  $S_1$ , the same as in the preceding, except that here 2A contributes the sex A nuclei, and  $S_1$  the sex B nuclei.

Fig. 11. Clon 3C is bisexual and a non-conidial offspring of 22  $\times$  34. Its nuclei are somewhat incompatible, as only a few perithecia form by selfing. An indefinite zone of ascocarps where the two mycelia, 3C and  $S_6$ , meet.

Fig. 12. Clon 3C  $\times$   $S_1$ . The same as the preceding except the sexes taking part are reversed. Very few ascocarps form by selfing. Hybrid ascocarps formed at the line of meeting. At the left two rows of fruit bodies along the line of meeting.

## PLATE 7

Fig. 13. Another culture of 3C  $\times$   $S_6$ . Note two rows of ascocarps where 3C meets  $S_6$ . The ascocarps in the upper row were proved to have been formed by 3C through selfing. Those in the lower row were hybrids 3C  $\times$   $S_6$ .

Fig. 14. Distribution of ascocarps when 3C is grown alone.

Fig. 15. Unisexual races 22 and 34 mated. Ascocarps formed in characteristic pattern on the area occupied by 22 which is sex A. Compare with fig. 17.

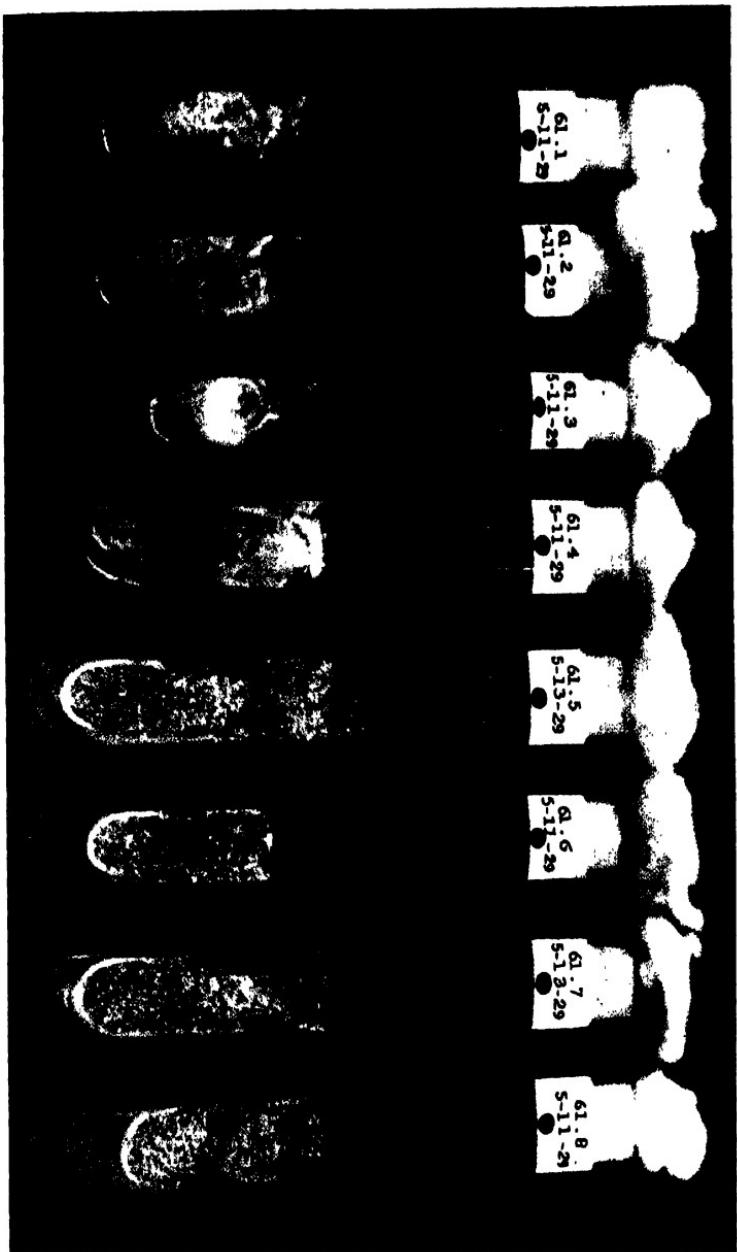
Fig. 16. Two hermaphroditic races, *tetrasperma* and 5M', each self-fertile. No line of ascocarps along the line of meeting.

Fig. 17. Two unisexual strains S<sub>1</sub> and S<sub>6</sub> of *tetrasperma* species, grown together, form ascocarps only on the S<sub>6</sub>, sex A, side.

Fig. 18. Two totipotent hermaphroditic races, 2G and 1A, cross and produce a line of large ascocarps along the line of meeting.

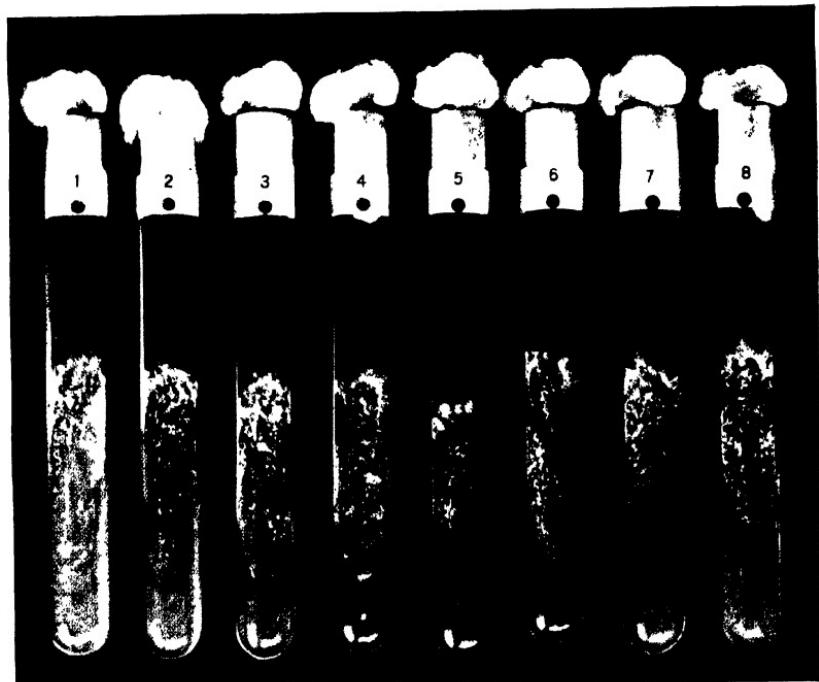
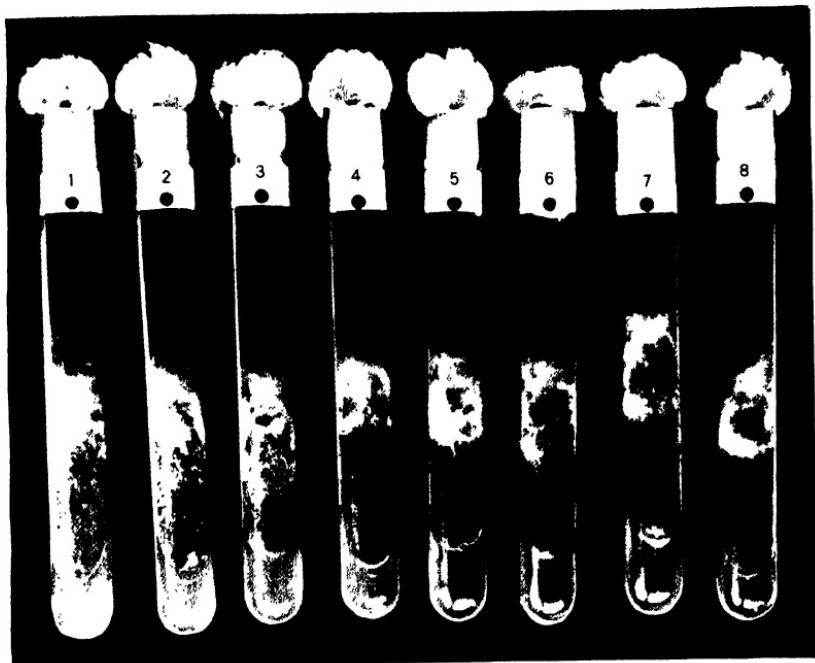
#### NOTE

In this paper the terms hermaphroditic, homothallic, haplomonoeious and bisexual are all used in the same sense to indicate a mycelium having two kinds of nuclei as to their sex, or whatever it is here that corresponds to sex. Kniep's expression shortened to "mikto-haplo-monœious" might be preferable.

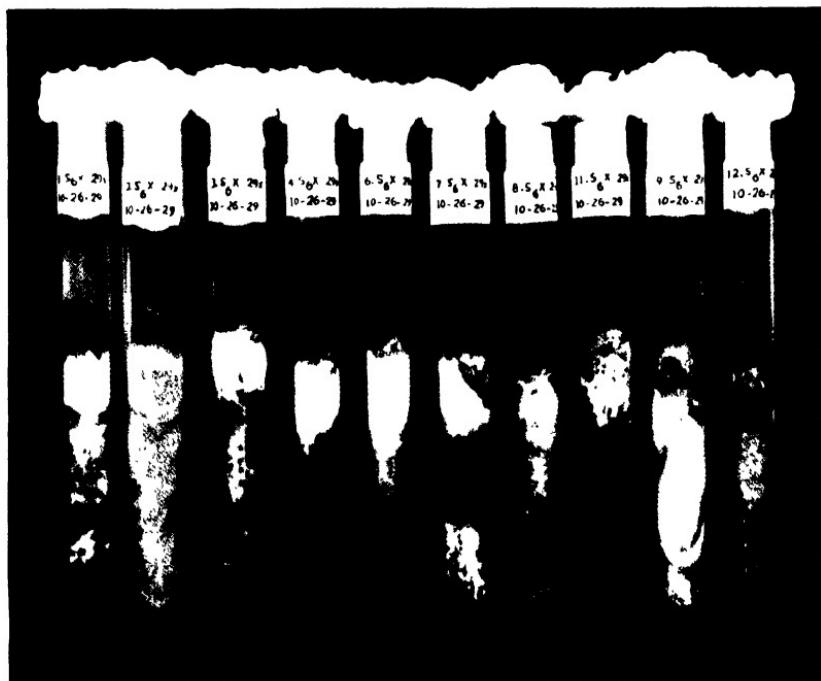
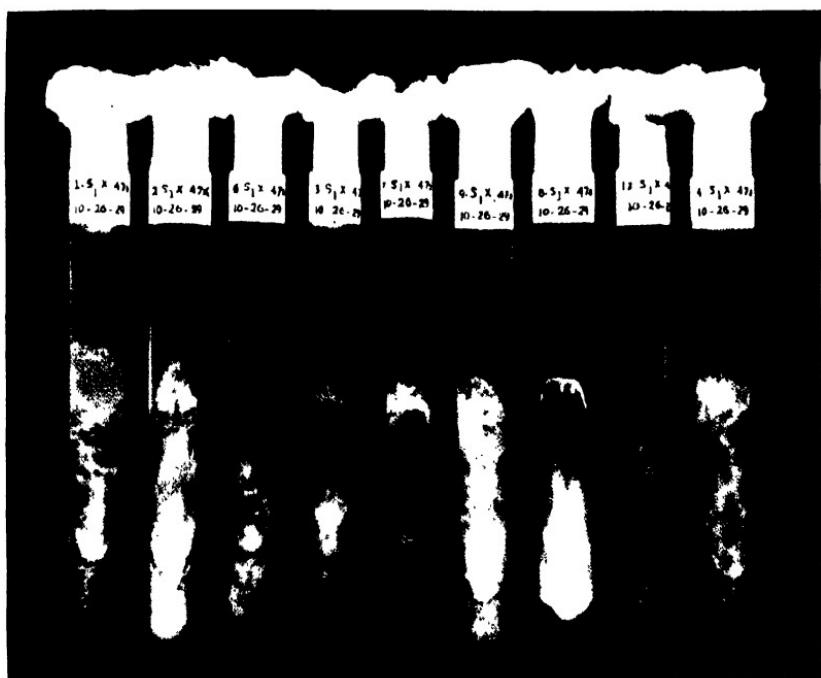


SEREGREGATIONS IN *NEUROSPORA SITOPHILA*



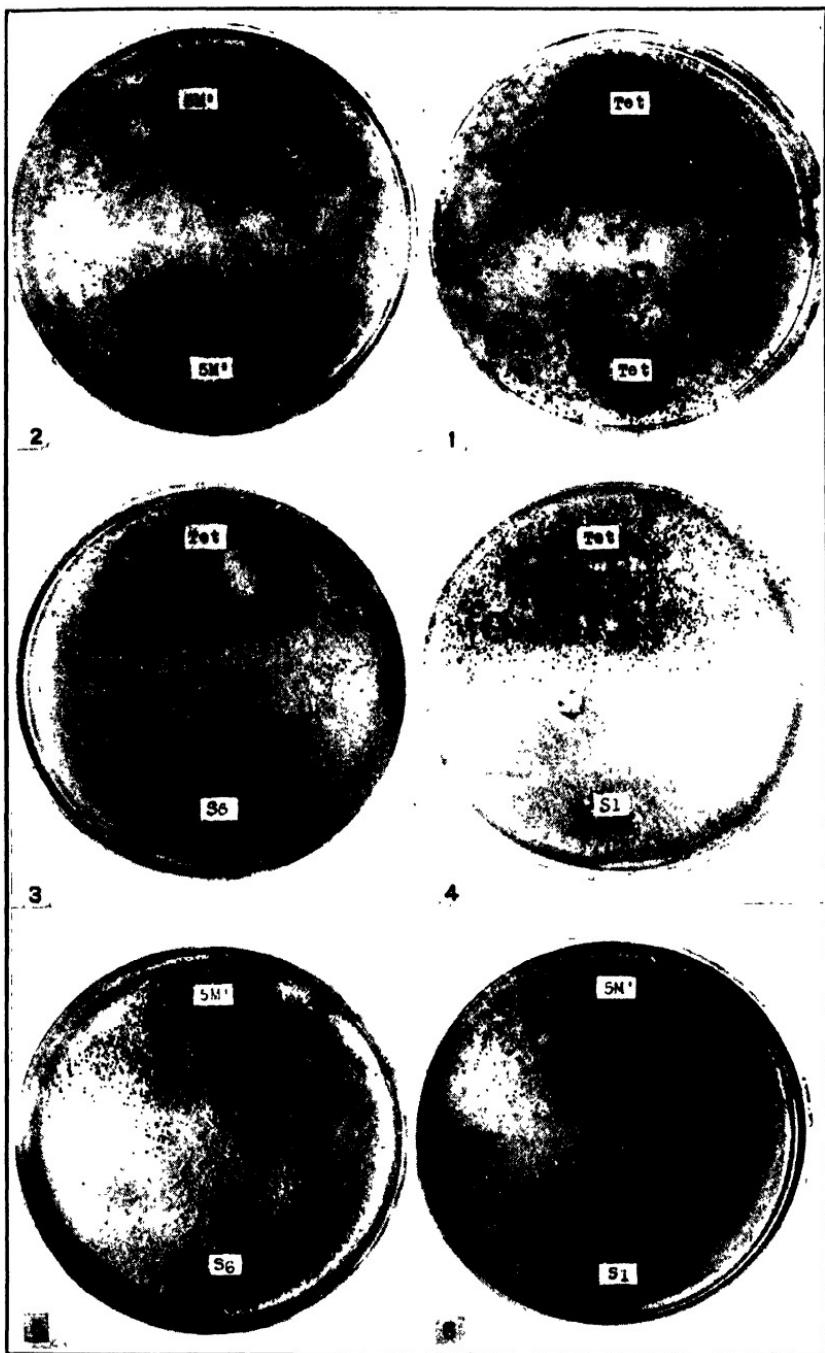
ALBINISTIC AND TYPICAL *NEUROSPORA SITOPIHLA*





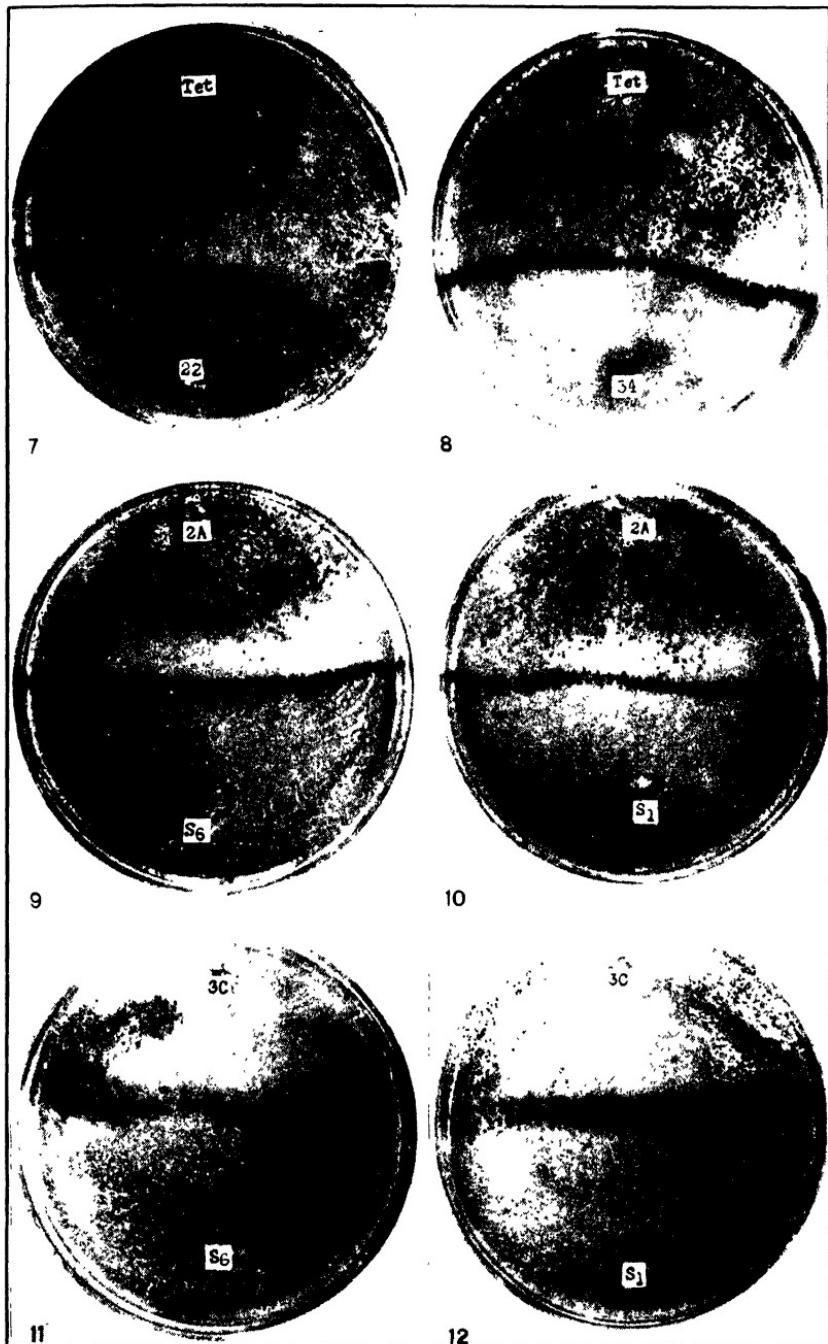
NEUROSPORA-HYBRID OFFSPRING





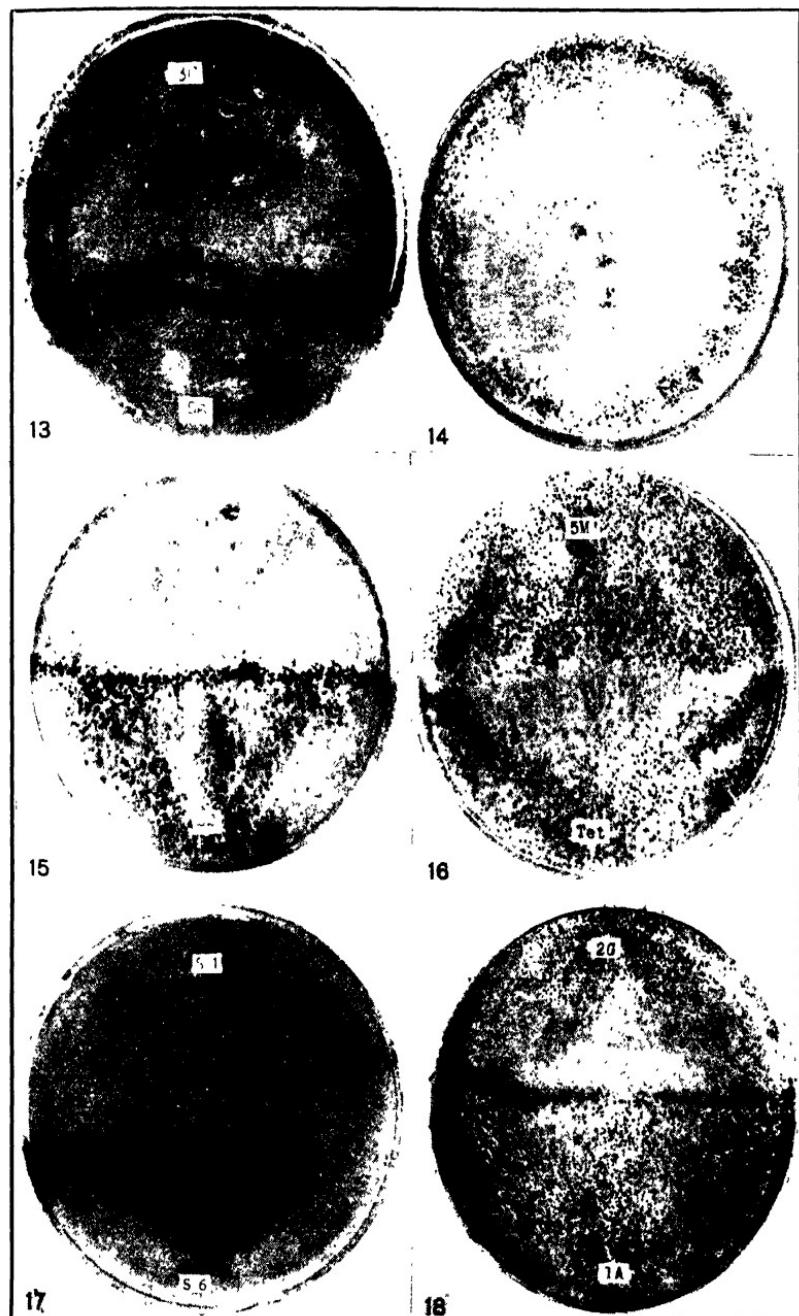
PAIRED CULTURES OF NEUROSPORA STRAINS





MATINGS OF NEUROSPORA HYBRIDS





MATINGS OF NEUROSPORA HYBRIDS



# STUDIES ON ASCOIDEA RUBESCENS

## I. HISTORY AND DEVELOPMENT<sup>1</sup>

LEVA B. WALKER

(WITH 5 TEXT FIGURES)

There are few groups of fungi concerning which students of fungi are more curious than those commonly included in the Hemiascales. This curiosity exists because these forms show characteristics of both Ascomycetes and Phycomycetes but differ widely from typical forms in either group. Most of the forms placed in the Hemiascales are rarely found. Workers who study them find it difficult to trace satisfactorily their life histories. This has been especially true of *Ascoidea*.

### HISTORICAL

The genus *Ascoidea* was established for *A. rubescens* by Brefeld and Lindau. The fungus was collected by Lindau during September and October growing in the sap flowing from felled beeches in the royal forest at Wolbeck. Brefeld (1) studied the fungus and in 1891 gave a comprehensive description of its gross morphology. Popta (4) carried further these studies and added observations upon its nuclear condition. These two papers have formed the basis of our general knowledge of the genus. The fungus has a coarse, branching mycelium which is abundantly septate at all times. On this mycelium abundant conidia and sporangia<sup>2</sup> develop. The sporangia which freely proliferate contain many small, walled spores imbedded in a granular, oily slime. The spores which are discharged from the sporangium at maturity have a characteristic hat-shape. Brefeld says they are formed in twos, but this is denied by Popta (4). Popta (4) found a multinucleate condition constantly present in vegetative and reproductive structures. Thus we have a fungus with a

<sup>1</sup> Cytological observations will be taken up in a second paper.

<sup>2</sup> The term, sporangia, used by Brefeld for these structures is continued but its use does not imply a morphological significance.

mycelium resembling that of Ascomycetes, forming sporangia in a Phycomycetous manner, but containing walled spores imbedded in a conspicuous slime that has at least a resemblance to the epiplasm of the Ascomycetes.

A second species of *Ascoidea*, *A. saprolegnioides*, was described by Holtermann (2) from materials collected from slime fluxes on various trees in Java. He distinguished this species from *A. rubescens* by the facts that (a) the proliferating growth forms one or more cells before forming a new sporangium and that (b) the sporangiospores lack the hat-shape.

No other papers dealing with *Ascoidea* seemingly appeared up to 1926 when Lohwag (3) published an extensive paper upon the homologies of the conidia of *Ascoidea*. This paper is apparently based upon the examination of mounts made by Schiffner from materials that the latter had found on maple trees in the Proter of Vienna. It contains little new information. However, Lohwag concludes that *Ascoidea* is in every respect a Phycomycete.

The last and only other paper is a brief summary of results obtained by Varitchak (5), a student of Dangeard. He describes the occurrence of two specially differentiated nuclei in the sporangium. These differentiated nuclei fuse but all other nuclei originally in the sporangium degenerate. The nuclei for the spores come from the repeated divisions of the fusion nucleus in a manner very similar to that described for *Dipodascus albidus*. He, therefore, concludes that *Ascoidea* is an Ascomycete. Thus the two latest workers dealing with the fungus come to absolutely contradictory conclusions as to the nuclear behavior in the sporangia and as to the relationships of *Ascoidea*.

Apparently the four collections of *Ascoidea* mentioned are the only ones recorded. No record of the occurrence of the genus in North America has been found. For this reason the author's collection of this fungus, August 8, 1927, from a slime flux on an elm tree at Ithaca, N. Y., becomes of especial interest. In spite of the fact that the fungus is unreported it seems probable that it is not uncommon in this country. During the fall of the same year many collections from similar slime fluxes on elm in the vicinity of Lincoln, Nebraska, showed remnants of *Ascoidea*.

which had evidently been killed by the extremely hot, dry weather that had prevailed during the early part of September. For similar reasons none was found here during the summer and fall of 1928, *i.e.* there were no slime fluxes in which it could grow. The early spring of 1929 was damp and cool and slime fluxes began to develop upon elms, but as *Ascoidea* had always been found in late summer no examinations were made. Later an examination of these slime fluxes, then partially dried, showed the fungus had again been present but had been killed by heat and drought. Proliferations up to seven were often observed on such hyphal remnants. Occasionally one or more typical hat-shaped spores were found that had failed to escape from the sporangium and had been held between the wall of its sporangium and that of the proliferating sporangium. Typical conidia were also observed. All attempts to secure cultures from Lincoln collections were unsuccessful. The mycelium seemed dead and the spores failed to germinate.<sup>3</sup>

#### MATERIALS

The slime flux from which *Ascoidea* was collected for these studies occurred on the south side of a large elm tree situated at the foot of the "Forest Home path" leading down from the campus of the New York State College of Agriculture at Cornell University to the highway southeast of Beebe Lake.<sup>4</sup> The

<sup>3</sup> On June 11, 1930, just after the manuscript of this paper had been forwarded to the publisher, an abundance of very luxuriously developed *Ascoidea* was found in slime fluxes on elm trees near the College of Agriculture at Lincoln, Nebraska. May and June, up to that date, had been unusually cool and damp. The slime fluxes had developed from imperfectly healed pruning or other injuries on the trunks of the trees. In most cases the growth resembled in appearance the original collection. On several trees, especially on pruning injuries, the *Ascoidea* developed in definite mats 3-4 cm. broad, 7-8 cm. long, and about 1 cm. thick. These mats had evidently developed radiately. Actively growing hyphae, conidia, and sporangia were largely limited to surface areas. Hyphae and sporangia were commonly so definitely oriented and they gave the appearance of a vaguely defined hymenium. In several cases *Fusarium* was found growing with the *Ascoidea*.

<sup>4</sup> Dr. D. S. Welch, Cornell University, said that the tree had been injured about five years previously by two ax strokes. The upper of the two wounds healed normally but the lower, about four feet above the ground, developed the slime flux.

fungus continued its growth during the remainder of August, which was unusually cool and dry. During most of that period the area covered by the sap flow extended 12–18 inches below the top of the wound but refuse on the surface of the tree indicated that at times it must have extended almost to the base of the tree and covered a wedge-shaped area 4–6 inches across at the lower end. The flow of sap seemed to be confined to a strip a few inches in length at the top of the slime flux and to come from a rift in the bark hardly a half inch wide at the widest part. The bark below showed a continuation of the rift down to within about three inches of the ground. The bark along this rift, which seemingly showed remnants of slime flux on its surface, was much rotted and filled with all sorts of microscopic animals. *Ascoidea* was confined to a few inches at the top of the slime flux and the youngest and most vigorous growth was found in the upper half inch. The young growth was of a much lighter color than that below. The slime flux occupied by *Ascoidea* looked about the same as that lower down on the tree but when touched the part containing *Ascoidea* was readily seen to be made up of very coarse hyphae having much the texture and appearance, except for color, of a stunted growth of *Cladophora*. This filamentous, matted part of the slime flux gradually merged into the lower part of the slime flux which was an amorphous, granular slime. Mixed with *Ascoidea* in the upper part and filling the granular slime below were found myriads of microscopic animals, larvae, nematodes, etc., as well as yeasts and bacteria. No other filamentous fungus was observed in the slime nor did any develop in isolation cultures made from the *Ascoidea*.

The day following the finding of *Ascoidea* in the slime flux practically all of the fungus was removed from the tree. Isolation cultures were made, materials fixed in various solutions for sectioning, parts preserved in formal-acetic-alcohol and parts dried for herbarium purposes.<sup>5</sup> The fungus continued its development and two subsequent collections were made during August but the flux appeared to be drying when last observed by the writer near the end of August. It dried soon afterward,

<sup>5</sup> Specimens have been deposited in the herbaria of Cornell University, Harvard University, the University of Nebraska, the New York Botanical Garden, and the Missouri Botanical Garden.

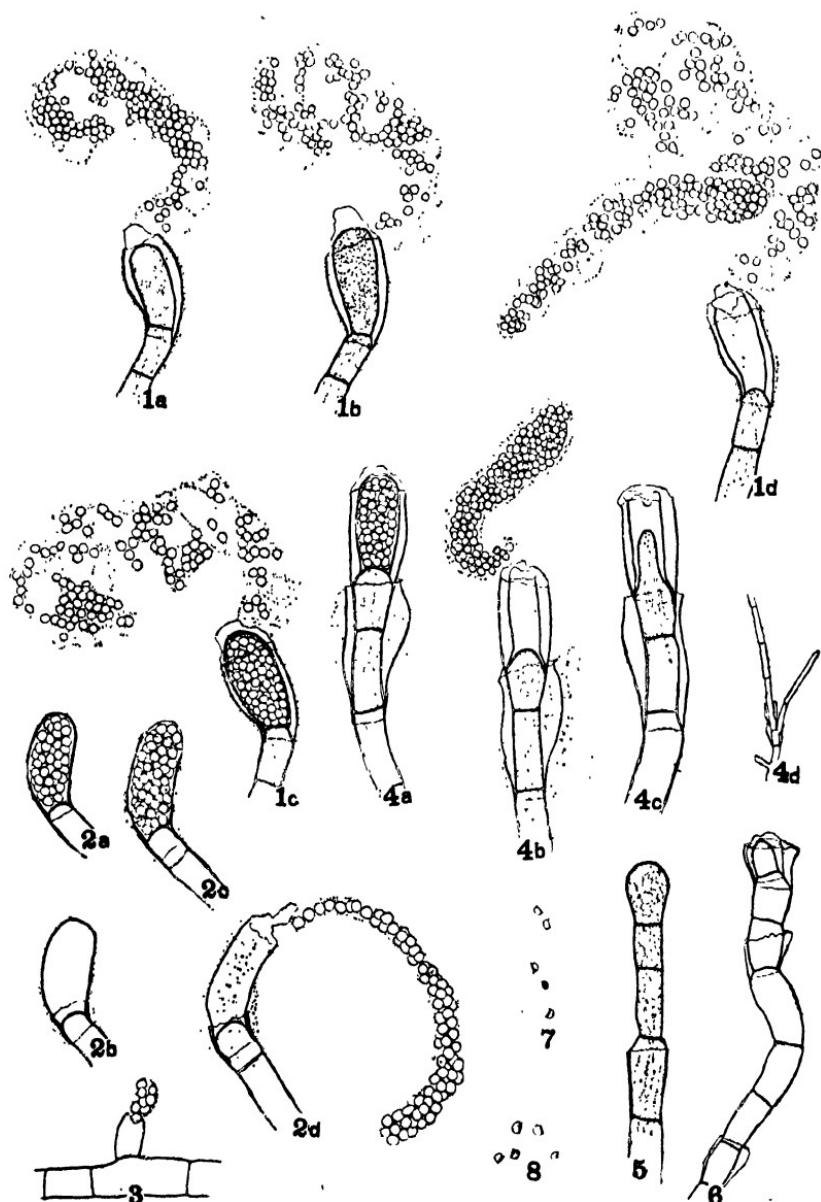
according to reports, and the development of *Ascoidea* in it has not since been observed.

#### OBSERVATIONS ON ASCOIDEA FROM SLIME FLUX

Materials taken directly from the slime flux were mounted in water in hanging drops. The mycelium consists, as described by earlier workers, of coarse, septate, branching hyphae (FIG. 9) in which the protoplasm shows conspicuous and characteristic longitudinal meshes (FIGS. 1-5, 10, 13). On the ends of some branches sporangia (FIGS. 1-6) might be present or develop while on others conidia (FIGS. 10-13) were found. Growth in hanging drops would usually continue about a week, gradually decreasing unless additional nutrients were added. When some nutrient was added very rapid growth would be resumed for a day or two but bacteria would develop so abundantly as to destroy the culture after that time.

*Sporangia*.—The development and discharge of many sporangia were followed in these water hanging drops. A few of these are shown in figures 1-6. The sizes and shapes of the sporangia were extremely variable. The most common type is shown in figures 1c, 2a, and 4a. Such sporangia developed on the ends of hyphae which widen slightly toward their tips (FIG. 4a). Smaller sporangia occurred and were usually observed as the nutritive supply became reduced. Many sporangia also arose laterally from hyphae (FIG. 3). In most of the sporangia the spores were so numerous that the number could scarcely be estimated, and in the smaller sporangia the numbers were extremely variable. The smallest sporangium observed (FIG. 3) contained eight spores. Successively larger sporangia contained 11, 16, 23, 84, etc., spores, the number varying according to the size of the sporangium.

The development of a sporangium usually required about three days. Figure 1a shows a sporangium about a day old, which is proliferating through an old sporangium, and the mass of spores that had been discharged from the old sporangium. At this time the young sporangium appears as a cell densely filled with highly refractive protoplasm. Figure 1b shows the same sporangium 16 hours later. The sporangium has enlarged and the protoplasm



Figs. 1-8. Sporangia as observed in mounts made from the slime flux.  
 1. Development at end of a sporangium-bearing hypha during 2 days and changes in discharged spore masses: 1a, a young sporangium proliferating through an empty one and the discharged spore mass 4 p.m.; 1b, beginning of spore formation in the developing sporangium and increase in vacuolization

has taken on a foamy consistency which characteristically precedes spore formation. During the next 24 hours the spores became rounded and looked mature (FIG. 1c). The actual discharge was not observed in this case but figure 1d shows its appearance 6 hours later.

The details of a discharge were probably most clearly observed in the sporangium shown in figures 2a-2d. The first figure (2a) shows a mature sporangium that had proliferated through either a very small sporangium, or more probably a broken cell. The wall of a sporangium is made up of three layers, a thin outer, a thick middle, and a thin inner layer. The outer layer is evidently somewhat gelatinous and in mounts made from the slime flux always contained many minute granules of debris. Where present these granules have been shown by stippling. (These granules are absent around sporangia grown in pure cultures.) In figure 2b the rupture of the outer layer of the wall near the tip of the sporangium may be seen. It occurred 45 minutes after the condition shown in "2a" and was easily seen because of the granules in the outer slime. The ruptured layer is seen on each side near the tip. It was 4½ hours later that the thick middle wall of the sporangium ruptured (FIG. 2c) and for two minutes the bulging spore mass was held by the delicate elastic third and innermost layer of the sporangium wall. When this inner wall gave way, in this case and all other cases observed, the spore mass rushed out rapidly at first, more slowly later, into the surrounding water (FIG. 2d). The spores emerging during the latter part of the discharge came out in almost single file. The various phenomena recorded here were repeatedly observed on various spo-

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of spore mass 8 a.m. the next day; 1c, continued development 8 a.m. the second day; 1d, appearance after discharge of second sporangium 2 p.m. of the second day. 2. Details of discharge of sporangium: 2a, mature sporangium 12 m.; 2b, rupture of outer wall near apex (content not shown) 12:45 p.m.; 2c, rupture of thick middle wall 5 p.m.; 2d, rupture of inner wall and discharge of spore-mass as it appeared 2 minutes later. 3. A small lateral sporangium. 4. Development at end of a sporangium-bearing hypha: 4a, afternoon; 4b, 9 a.m. following day (transferred to bouillon and position changed); 4c and 4d, vegetative growth resulting. 5. Protoplasm in a hypha, the terminal cell probably a young sporangium. 6. Series of small empty sporangia through which proliferations had occurred. 7. Spores taken directly from slime. 8. Spores after standing in bouillon. 4d X 39; others X 200.

rangia but not enough were watched continuously to be sure whether the lengths of periods between the rupturing of the three layers in the wall were typical or not. It seems these periods may be very variable. Discharged masses from large sporangia were usually the shape of a curved club, while those from smaller sporangia were more globular. The time occupied by a discharge was only a few seconds. A discharge from a sporangium of *Ascoidea* appears much like the discharge of zoospores from the sporangia of water moulds, such as *Achlya*, except for the presence of the densely granular slime between the spores. All of the spores were usually discharged in one mass which at the end of the discharge lay at a little distance from the mouth of the sporangium. Occasionally, however, a few spores became separated from the discharging mass and failed to get out of the sporangium. Such spores seemingly never escape from the sporangium and remain permanently between their old sporangium wall and the growth proliferating through it.

On the sporangium-bearing tip, shown in figure 1, the new sporangium was formed within the old one but in a majority of cases observed the proliferating hypha formed one or more vegetative cells before forming a second sporangium as shown in figures 4-6. Figure 4a shows one nearly mature sporangium and two empty ones. After the discharge of the first sporangium two cells were formed before the formation of the second sporangium while the third was formed directly within the second. After the discharge of the third sporangium the specimen was transferred to bouillon and the position of the sporangium changed. The nutrient medium caused a return to vegetative development with the results shown in figure 4c, 4d.

Figure 5 shows three vegetative cells and what is probably a primordium of a sporangium. Such broadened terminal cells with dense protoplasm and rounded vacuoles usually develop into sporangia but in some cases vegetative growth is resumed. Other workers have found it impossible to distinguish sporangium-bearing from conidium-bearing hyphal tips but in this material sporangium-bearing hyphae were broader than others and toward the apical end usually increased slightly in diameter. In many cases the cell just below the sporangium was sufficiently different

from the other vegetative cells to remind one of the sufflatory cells associated with oögonia in *Oedogonium*.

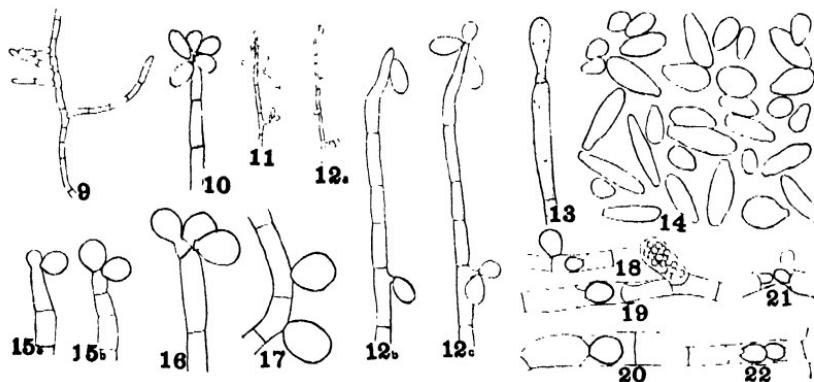
The spore mass as discharged from the sporangium consists of spores imbedded in a homogeneous, oily slime (FIGS. 2d, 3, 4b). Within a few hours vacuoles begin to appear in the slime. The vacuoles increase in number and size apparently by the absorption of water and thus gradually the spore mass is enlarged and the spores separated. Often the vacuoles become so large that they break open, leaving peculiar points and circular depressions in the outer part of the slime. These changes are illustrated in the spore masses shown in figure 1a-1d. The spore mass to the left in figure 1d shows the beginning of the vacuoles and the spore masses in figure 1a-1c illustrate the successive changes during 2 days in the same spore mass.

The spores as they lie in a spore mass appear as small rounded bodies of quite uniform size. When critically examined they appear as in figure 7, flattened on one side and having a hyaline projecting rim around the flattened side. These spores, commonly known as "hat-shaped," are characteristic of *A. rubescens* and a number of other Hemiascaceous fungi such as *Endomyces fibuliger*, *E. decipiens*, *Willia anomala*. Spores in bouillon or water enlarged somewhat (FIG. 8) but did not germinate. In young spore masses the spores are held so firmly in the slime that it is almost impossible to separate them but as the slime becomes more and more vacuolate it is often possible to separate them into smaller groups. No germinations of sporangiospores were secured from materials secured directly from the slime flux.

Microchemical tests show that most of the vegetative cells are densely filled with glycogen and many also contain oil. Young sporangia contain much glycogen and protein while mature sporangia contain little if any glycogen. No glycogen could be detected in discharged spore masses. The slime around the spores is an oily substance containing many granules, the nature of which could not be determined. They probably represent waste materials.

**Conidia.**—Conidia were abundant on all materials examined but no detailed studies have been made of them. Three types of conidia have been recognized by previous workers: cylindrical;

oval; and rounded, thick-walled, resting conidia. Although the three types are easily recognizable, there are such complete intergradations between types that such a classification seems useless. The oval and cylindrical types are commonly developed at or near the surface of the slime flux, while the rounded thick-walled conidia are formed on older, submerged hyphae. The conidia are most abundantly produced apically at the tips of main branches or on short lateral branches (FIGS. 10, 11). The conidia do not readily fall and conidiophores bearing dozens of conidia were commonly seen in mounts as have been well illus-



FIGS. 9-22. Mycelium and conidial formation. 9. Typical mycelium from slime flux. 10-13. Formation of conidia on younger hyphae. In 10 protoplasmic structure is indicated. 11. Variations in size and shape of conidia still adhering to conidiophore. 12. Development of conidia in hanging drop: 12a, conidium-bearing hypha; 12b, tip of same hypha enlarged; 12c, same tip 2 days later. 13. Attachment of a young conidium. 14. Variations in size and shape of conidia seen in a single field of the microscope in a 10 day old culture. 15-22. Development of conidia on older hyphae: 15, globular conidia on end of hypha; "b" 16 h. after "a"; 16 and 17, thick-walled conidia on old hyphae, developed apically in 16 and laterally in 17; 18-22, development of thick-walled conidia internally and laterally on old hyphae. Figs. 9, 11, 12a  $\times 39$ ; 10  $\times 200$ ; all others  $\times 215$ .

trated by Brefeld (1). The conidia are formed on the ends of hyphae that continue at once apical growth, pushing the conidia aside, and proceeding to form other conidia. Figure 12a shows a young branch bearing oval conidia. Its tip highly magnified may be seen in figure 12b, while 12c shows its appearance two days later in a hanging drop. Characteristically there is a narrowing of the spore just above the point of attachment, so

that a sort of stalk is formed at the basal end as shown in figure 13. The development of globular conidia is shown in figure 15a, 15b, the second drawing showing development 16 hours later. Another case is shown in figure 16. Such conidia have heavier walls and are formed after active growth has ceased.

Conidia are not only formed on the tips of branches but may be formed laterally (FIG. 17) or internally (FIGS. 18-22) from older, submerged hyphae. Such conidia are usually heavy walled and often the wall is somewhat colored. So far as observed they are of the rounded, resting type. The drawings for illustrating this type of development were largely made from cultures of *Ascoidea* but the same types were abundantly present in materials taken from the slime flux. Variations in the size and shape of detached conidia observed in a single field of the microscope are shown in figure 14.

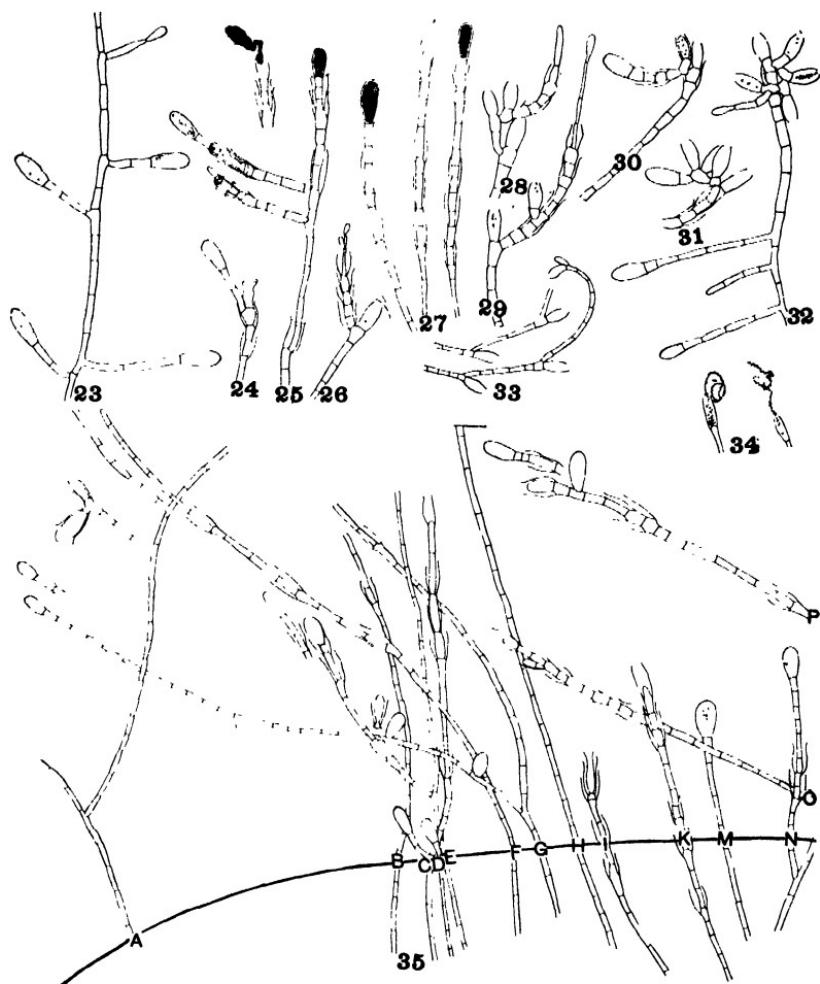
#### CULTURES

Seventeen pure cultures of *Ascoidea* were secured from the original materials. Some of these were from single conidia, others represent growth from several conidia and still others were secured from mycelial fragments. Growth in all cultures was similar. Because of the difficulties encountered in securing the germination of sporangiospores no cultures have been secured from this source. These cultures have been maintained and have furnished materials for the further studies here reported. Vegetative growth is good on all media tried but best in liquid media. The most satisfactory cultures have been on elm twigs nearly submerged in nutrient solutions. In these the fungus could grow over the wet surface of the twig, securing at all times ample moisture and exposure to the air. Of the many nutrient media tried the most satisfactory has been 0.3 per cent beef extract + 3 per cent dextrose in an elm twig decoction.

All cultures formed a multitude of cylindrical and oval conidia wherever actively growing mycelia reached the surface. The conidia were formed either in the air or in liquid near the surface. Thick-walled, globular conidia were formed in older cultures on submerged hyphae. If the entire culture was submerged no other reproductive structures appeared. Rarely were sporangia developed and when produced were usually quite abnormal.

They only formed in old, depleted cultures. In some cases normal proliferations up to seven were observed. Often the spores appeared to have been neither normally developed nor discharged, as the sporangia contained remnants of spores in a surrounding slime. If the development and discharge were only slightly abnormal so that most of the contents escaped the sporangium, proliferations took place normally as shown in figure 25. If very abnormal, a true sympodial development resulted. Figure 26 shows a commonly observed condition where the terminal sporangium appeared blighted and a branch just below it had continued the sporangial development, forming and emptying 4 sporangia and finally proceeding to the formation of conidia. The extreme of this type of development is shown in figures 28-33. The most plausible explanation for this variation is that lack of moisture prevented the normal development of the sporangium and growth was resumed below. The development of normal appearing sporangia below the aborted ones, as shown in figure 32, would suggest this. Similar development was repeatedly observed in slide cultures, as will be described later, that had become too dry. The presence of these sympodially developed sporangia adds weight to Lohwag's (3) contention that proliferations are modifications of the sympodial type of development as in this case the sporangia and conidia follow the same developmental sequence. All who have studied the fungus have held that conidia and sporangia are modifications of the same structure.

Many types of cultures were tried in the vain hope that an abundant development of sporangia might be secured. Brefeld (1) and Popta (4) seemed to have had little if any more trouble in securing abundant sporangia in old cultures than conidia in young cultures. These authors do not give any details as to how the cultures were handled. Various media, and changes from high nutrition to low nutrition, etc., were tried but in no case were sporangia ever developed sufficiently in pure cultures to supply materials for sporangial studies which were most desired. The first real success was observed when reexamining a mount made three days previously from an elm twig culture that showed a few sporangia. The mount had been placed in a



Figs. 23-35. Development of sporangia in cultures. 23. Hypha bearing 4 sporangia and one conidium developed beyond the edge of the cover glass in a mount held in a moist chamber for 2 days. 24-27. Commonly observed types of proliferations. 24. New terminal branch developed after one has blighted. 25. Left, proliferations through an incompletely emptied sporangium. (The insets show development one day later; the discharge of the terminal sporangium is somewhat abnormal.) 26. Aborted terminal sporangium and development of new sporangia below; the last proliferating hypha having developed conidia. 27. Three hyphae showing the most common types of proliferation and development of sporangia. 28-33. Variations in development of sporangia observed in old cultures, 32, showing a combination of normal and abnormal types of development. 34. Abnormal discharges of spores in long worm-like threads as described by Brefeld. 35. Development of sporangia at edge of cover-glass (heavy line). All insets show development 2 days later. Discharged spore masses were omitted. All  $\times 50$ .

Petri dish on wet filter paper, hoping that more sporangia might develop. The original sporangia had matured normally and discharged their spores but no more had developed under the cover glass. The mount was about to be destroyed when it was observed that on a few hyphae, that had extended beyond the cover glass, over 20 young sporangia had developed. Figure 23 shows a part of one of these hyphae. During the next few days these sporangia matured, discharged their spores, proliferated, etc., in an entirely normal manner. Other mounts were made from the same culture tube and behaved similarly but mounts from other media and other cultures behaved variously and diverse results were secured in each series of experiments. In a long series of experiments abundant sporangia were secured at one or more times from all cultures used but no conditions were found that could be manipulated so as to secure uniform results. In these experiments mounts in nutrient media and in water, mycelium in thin films of water on slides held in moist chambers, and mycelium in thin films of water in Petri dishes and in hanging drop cultures were used. The most dependable development of sporangia was secured when masses of actively growing mycelia from a culture tube were: (a) washed in several changes of water; (b) let stand in thin films of water in a Petri dish for several days; (c) mounted in abundant water so that some mycelium was near or beyond the edge of the cover glass and placed in a moist chamber; and (d) let dry daily for several days till all extra water at the edge of the cover was gone and then rewatered. By this method 100 per cent of mounts in some series showed abundant sporangial development while in others almost no sporangia appeared. This variation may have been due to the age and vigor of the original culture, to the degree of drying given the mounts, to inherent tendencies, or to a combination of these factors. Old depleted mycelium exposed either in thin films of water in Petri dishes or at the edge of cover glasses in moist chambers usually developed sporangia.

The formation of sporangia almost directly from germinating conidia (FIGS. 53-57) and mycelial fragments (FIG. 58) has been secured repeatedly under quite different conditions. In each case mycelium from a culture tube had been washed on several suc-

cessive days to remove the nutrient media and left standing for several weeks on the surface of moist filter paper, much rotted willow wood in water, or in thin films of water in Petri dishes. Conidia, and occasionally mycelial fragments, washed from such extremely depleted cultures and left standing deeply submerged in water germinated and many gave rise to sporangia. Conidia washed from the same original cultures and left standing in water during the same period or even much longer never germinated. The long exposure to the air seemingly made such germinations possible.

Feeling that development of sporangia might be definitely traceable to an oxygen requirement mycelium was placed in water and in nutrient media in flasks and connected in series by means of Y-tubes with an aspirator so that air was constantly bubbling through the cultures. In no case did sporangia develop abundantly.

As a result of many trials the following general conclusions as to the conditions essential to the development of sporangia seem evident:

1. That a great reduction in the available nutrition is essential.
2. That sporangia develop only on hyphae that are at or near the surface of the water or from germinating spores that have had long exposure to the air.
3. That alternate drying, to a point as dry as can be attained without injury to the hyphae, and wetting are favorable.

Thus it seems that *A. rubescens* has become so adapted to the fluctuating conditions that must prevail in a slime flux that it must have similar conditions to stimulate it to development of sporangia.

The reactions of the fungus to air are very striking. Very little growth takes place under a cover glass. Stray hyphae near the edge of the cover glass will send out many branches toward the edge of the cover, which will branch and rebranch until a perfect tangle of hyphae is formed just beneath the edge of the cover. Where the hyphae are smaller and copiously branched conidia usually develop abundantly beyond the cover glass; where the hyphae are larger and less branched sporangia develop. A characteristic portion of a sporangium-bearing growth at the

edge of a cover glass is shown in figure 35. The heavy line upon which the letters are placed represents the edge of the cover glass. In this case the distance between A-B and G-H was slightly shortened. Otherwise all actively growing hyphae are shown in position. The sporangium-bearing hyphae all grew from a mass of mycelium under the cover but near its edge. The insets show development that had taken place two days later. Spores were matured and discharged normally. No attempt was made to show spore masses, as repeated waterings and handling of the slide moved them from the places where they would normally have been found. Sporangia are stippled to make them more easily distinguishable but no attempt has been made to show cell contents.

Figures 24-27 and 35 are arranged to show the variations in sporangial arrangement ordinarily observed in such cultures. A sporangium-bearing hypha is first definitely recognizable as a branch which apically increases its diameter as it develops. Soon the end cell enlarges and the protoplasm becomes more dense (FIG. 35, D, M). Here, as in materials taken from the slime flux about 3 days is required for the development and discharge of a sporangium. After discharge a proliferating hypha grows into it which may form a cross wall at once and form a sporangium (as shown on the branch to the left, figure 25) or develop several cells before forming a second sporangium (figure 25, main hypha, 27, or most cases shown in figure 35). Here also sessile and lateral sporangia often occur (FIG. 35, G, P). It is not uncommon to find two proliferations ramifying the same sporangium (FIGS. 24, 35, O). If a terminal sporangium failed to discharge normally its spores, a sympodial development was observed (FIG. 35, A). Figure 35 shows discharged sporangia under the edge of the cover glass but none were ever observed forming or maturing there. The position of the cover glass had evidently been slightly changed by handling. All such mounts showed more or less contamination with bacteria, yeasts, and other fungi because of their repeated exposures to the air.

### MECHANISM OF DISCHARGE

Literature on *Ascoidea* abounds with discussions as to how the spores get out of the sporangium. The general conclusion reached in each case, however, was that the onward growth of the proliferating hypha was largely responsible for the discharge. Brefeld (1), however, observed that the intersporal slime tended to swell and that the inner layers of the wall of the sporangium gelatinized, and he suggested that these might assist in initiating the discharge but that eventually the spores were pushed out. Surely their materials were much less vigorous than the ones used in these studies or they actually failed to observe normal discharges. The discharges illustrated by Brefeld (1) resemble the ones shown in figure 34, an abnormality that was several times observed. In such cases the spores would have to be pushed out if they ever escaped. No case was ever observed where spores remaining in a sporangium were pushed out by a proliferating growth. If much of the spore mass was left in the sporangium usually no proliferation occurred. If only a few spores remained they were pushed aside instead of out during proliferation. The discharges illustrated in figures 1, 2, 4 are characteristic of all observed. These figures (as 1d, 2d, 3, etc.) all show that the spores had been entirely discharged before proliferation occurred. Normally there is little if any outward bulging of the intervening cross wall before the discharge. Where the cross wall does bulge (FIGS. 1d, 4a) it might function as a columella in initiating a discharge. It seems more probable that the discharge is largely brought about by the accumulated osmotic pressure due to the transformation of glycogen, abundantly present in the young sporangium, and absent in the mature sporangium, into compounds<sup>6</sup> with high osmotic pressure held in the intersporal slime. That the intersporal slime has an enormous power for water absorption is shown by its great expansion after discharge and by the appearance of many vacuoles (FIG. 1). The release of tension upon the rupturing of the sporangium wall is sufficient to force out the greater part of the spore mass and the remainder may be drawn out by the tenacity of the intersporal slime.

<sup>6</sup> Microchemical tests failed to indicate their identity.

The innermost layer of the wall of the sporangium also appears to aid in the discharge. Discharges observed in slide cultures show a clear, apparently gelatinous, expansion of the innermost layer of the wall at the time of the discharge which so nearly fills the lumen of the empty sporangium that only a narrow channel remains through the center of the sporangium. This was much more conspicuous in sporangia which discharged abnormally and where some of the content of the sporangium remained in the central channel as shown in figures 26, 29, 32, and 58. In completely emptied sporangia this channel is only visible when the light is especially carefully adjusted as both are perfectly transparent. This modification of the inner wall was not observed in materials taken from the slime flux but the granules covering the outside of the sporangium in such materials would have made its observation very difficult, if not impossible.

The above observations point to the conclusion that the discharge is primarily brought about by high osmotic pressure developed by substances in the intersporal slime. It is assisted by the gelatinization of the inner layer of the wall of the sporangium and often at least by the slight bulging of the septum from pressure below.

#### IDENTITY OF THE AMERICAN ASCOIDEA<sup>1</sup>

As previously stated, only two species of *Ascoidea* have been described: *A. rubescens* by Brefeld and Lindau (1), and *A. saprolegnioides* by Holtermann (2). Holtermann distinguished his species on two points of morphological difference: that several vegetative cells were formed following a proliferation before another sporangium and that the sporangiospores lacked the hat-shape. Brefeld both describes and figures (Figs. 18, 19) cases where vegetative cells were interspersed between sporangia on proliferating hyphae and Holtermann, cases where no vegetative cells intervened (Figs. 19, 21). This appears to be at best a fluctuating characteristic and should hardly be considered a valid reason for separating two species. Thus it seems that the only possible point of separation of the two species is the absence of hat-shaped spores in *A. saprolegnioides*.<sup>2</sup> The American

<sup>1</sup> Holtermann's figures suggest that his lenses may not have been adequate for observing delicate details. The "brims" characteristic of hat-shaped

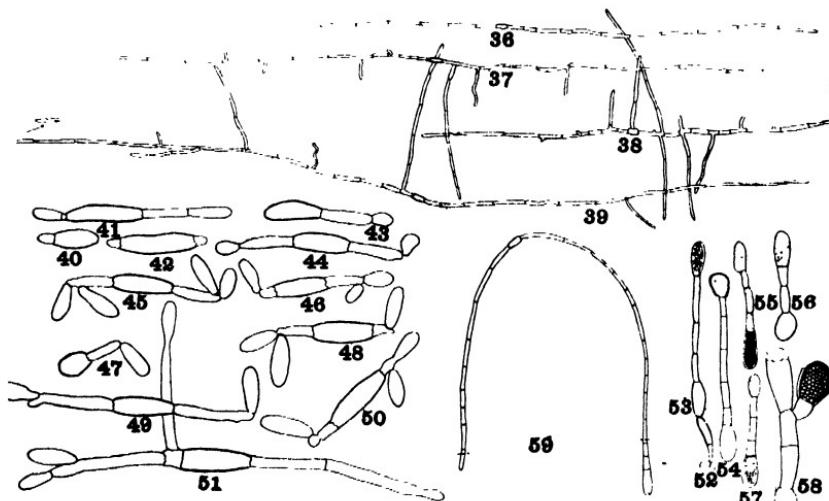
*Ascoidea* agrees very closely with Holtermann's description of his fungus except that it has hat-shaped spores. Since the hat-shaped spores are the most definite point for separating the two species it seems that the American form must be *A. rubescens* and that in this fungus extremes of variation in sporangial development may occur. All structures described by Brefeld have been observed in the American form, except a few abnormalities, and the occurrence of sporangiospores in twos which was denied by Popta (4) working with the same materials. *A. rubescens* is described as having a fishy smell which has not been observed in the American *Ascoidea*.

#### SPORE GERMINATIONS

One of the very striking characteristics of the spores, both sporangiospores and conidia, is that they rarely if ever germinate in cultures or in the slime flux where they are formed but germinate readily when spread out in a thin film of water or nutrient media, or on the surface of agar media.

*Conidia*.—The germination of conidia has been well described by Brefeld (1). In the author's studies extremes of variations were observed under varying conditions. Three extreme types with intergradations were observed. First, figures 36-39 show four conidia germinating on the surface of nutrient agar after 2 days. Under such conditions a branching septate mycelium develops at once. It was from such germinations that stock cultures were secured. Second, if conidia are placed in thin films of water the germ tubes at once proceed to form conidia (Figs. 40-51). The details of conidial formation show well here and are identical with conidial formation on vegetative hyphae. Such germinations were always observed around the edges of cover glasses where mounts were kept in moist chambers to secure sporangia, or where mycelia or water containing spores were spread out in thin films in Petri dishes or on slides. Elongated, oval, and thick-walled conidia all germinate alike except that the oval, or smaller, conidia usually develop only one germ tube spores are so transparent that they are not easily visible. It seems quite possible that if Holtermann's materials had had the hat-shape the fact might readily have been overlooked. Since this is the only definite distinguishing characteristic the validity of his species seems questionable.

(FIGS. 47, 52). A third extreme type of germination is illustrated in figures 53-57. In this case old mycelia from nutrient media had been placed on wet filter paper in a Petri dish in order that the possible development of sporangia might be watched more carefully. Many conidia and some sporangia developed. When the culture became depleted the spores were washed with water into a Petri dish and left standing. Several days later when examining the dish it was found that the conidia had germinated very abundantly. Some had germinated by directly forming



FIGS. 36-59. Germinations of conidia. 36-39. Four conidia germinating on agar medium, 2d at 23° C. 40-51. Germinations of conidia in thin films of liquid, 40-42 after 7 h, 43-51 after 24 h. 52-57. Germination of conidia from old depleted culture; 52, forming conidia and others forming sporangia. 58. Sporangia developing from a mycelial fragment. 59. A conidium germinating under edge of cover-glass (dotted line) and forming a sporangium beyond the cover-glass. 36-39 X 34; 40-51 X 188; 53-58 X 100; 59 X 44.

conidia (FIG. 52), others only germ tubes, and still others, about half, had formed sporangia on the ends of very short germ tubes (FIGS. 53-57). A few of the sporangia had discharged spores. Where fragments of hyphae occurred many of them had also proceeded to develop sporangia (FIG. 58). This type of germination was often observed under similar conditions. Intermediate between these extremes every gradation was observed. A short mycelium might develop and then proceed to form conidia, or

sporangia. Figure 59 shows a germinating conidium that lay just under the edge of the cover glass. Where submerged under the cover glass a tube resulted but when it reached the edge of the cover, as shown by dotted lines, sporangia developed. Conidial development was even more common in such cases. Very often under such conditions the hyphae branched profusely before reaching the edge of the cover glass and as the branches intermingled a mat of radiating hyphae, all having developed from spores lying just under the cover, is formed.

In many larger conidia, before germination, the protoplasm may appear as if endogenous spores were being formed, as in sporangia, but no endogenous spores matured. Similar observations were recorded by Brefeld (1).

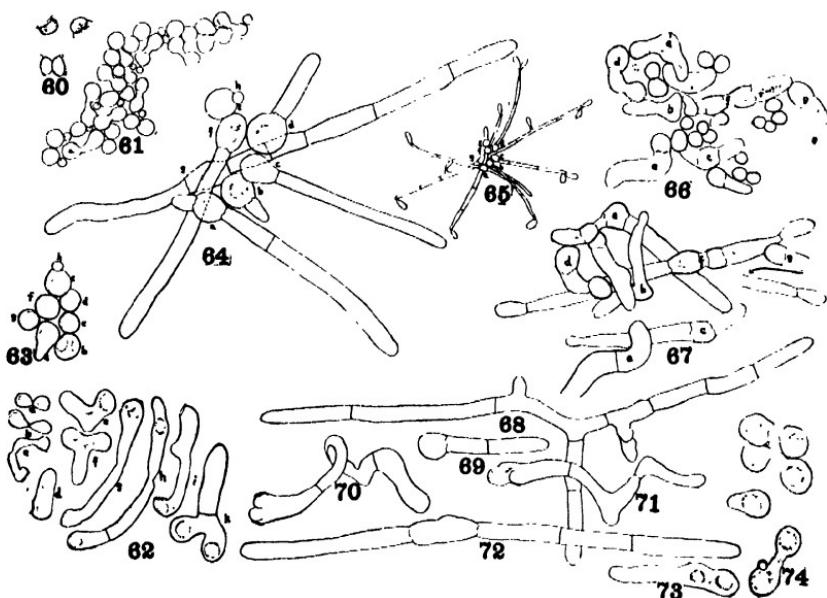
That an abundance of air is a prime necessity for conidial germinations was quite clearly shown by aspirating cultures. In unaërated cultures, the growth was confined to the original inoculum but in aërated cultures tiny colonies, evidently coming from germinating conidia, almost filled the nutrient media used.

*Sporangiospores*.—Extreme difficulty was experienced in studying the germination of the sporangiospores. While a few sporangia were formed in pure cultures it was almost impossible to find the discharged spore masses. The necessity of exposing cultures to the air to secure abundant sporangia almost always resulted in contaminations of various sorts. In spite of the fact that many attempts were made, using various methods, sporangia, without contamination, were rarely, if ever, secured. Even where seemingly clean spore masses were obtained the necessity for exposure to the air to secure germination of the spores again caused difficulty.

The removal of spore masses from the culture drops in which they had been discharged was very simple. Being held together by the oily slime, the entire spore mass, unless caught by hyphae, came to the surface of the liquid in which it was discharged. It was then relatively easy to reach with a needle, while watching under low power, and remove the entire spore mass as it would adhere to the needle. Some difficulty was experienced in removing the spore mass from the needle. It was almost impossible to free the spore mass from the needle in water but it could be

quite successfully removed by rotating the needle on the surface of a dry slide or cover glass. The slime was so tenacious that usually the entire spore mass adhered in a spot and it was difficult to break it up sufficiently to follow individual spores.

By removing spore masses, as described above, germinations of sporangiospores in considerably over a hundred spore masses were observed. Each sporangiospore, when critically examined, shows a dense protoplasm containing highly refractive granules



Figs. 60-74. Germination of sporangiospores. 60. Detail of sporangiospores. 61. Ungerminated (small) and germinating (larger) sporangiospores at edge of a spore-mass in water. Many are fusing in pairs. 62. Types of anastomoses commonly observed in nutrient media after 2-4 days. 63-65. Development in an isolated group of sporangiospores in nutrient solution where no fusions occurred, 63, after 2 days, 64, 3 days, and 65, 4 days. 66, 67. Development in an isolated group of sporangiospores in nutrient solution showing the most commonly observed types of germinations, 66, after 4 days and 67, 5 days. 68, 70, 71. Sporangiospore germinations on nutrient agar after 3 days and 69, 70, 73, after 4 days in nutrient fluid. 74. Detail of anastomoses. Figs. 60, 63, 64, 74  $\times$  286; fig. 65  $\times$  50; other figs.  $\times$  215.

and a large central vacuole (FIG. 60). Soon after sporangiospores are placed in a thin film of water they swell enormously and protrude germ tubes which usually fuse in pairs but rarely does germination progress beyond this point. Figure 61 shows

the appearance at the edge of a spore mass where the spores were sufficiently separated to be seen clearly. The small rounded bodies are ungerminated spores, the larger rounded cells, spores that have enlarged but not yet developed tubes, and the others the spores whose germ tubes have fused. During the germination the central vacuole of the spore becomes greatly enlarged. The vacuoles are indicated by dotted lines in the spores. Early stages in this fusion appear as shown in figure 62a and b, and a little later stages, more highly magnified as seen in figure 74. In such cases it is usually very difficult to observe the "brim of the hat." The fusion of tubes does not always occur between spores lying in close proximity to each other. A tube may pass by a number of spores and fuse with the tube of another at a distance. In such cases fusions appearing as shown in figure 62c, g, h, and i are common. In water usually the germinations proceed no further but occasionally short hyphae arise from the fused germ tubes as shown in figure 62d, e, f, i, and k. If, however, a nutrient solution was added the fused germ tubes gave rise to hyphae on which conidia develop promptly.

If, however, the spore masses are placed in a nutrient solution fusions of spores are less common. Figures 66 and 67 show such a mass; figure 66 shows the mass 4 days after the drop was made and 67 a day later. As it was a small mass of isolated spores it was hoped that it might be possible to follow individual spores but the growth of the germ tubes disarranged the spores so greatly that it was impossible to follow many of the spores. Only those that could be quite positively identified are shown in figure 67. Other spores, germinated in a nutrient solution (beef extract + dextrose + elm decoction), are shown in figures 69, 72, and 73. It may be seen that in some cases hyphae arise from fused spores (FIGS. 66, 67a, f, 72-73) and in other cases from individual spores (FIGS. 67b, c, e, g, 69). Regardless of their origin the resulting hyphae at once proceed to form conidia exactly as did tubes arising from conidia.

Figure 63 shows another isolated group of 8 sporangiospores after 2 days in a nutrient solution. One spore "h" did not swell. The others have all enlarged more or less and "a" has started to develop a germ tube. In figure 64 the development during the

next 24 hours is shown. All of the enlarged spores but "e" have developed germ tubes. A day later figure 65, "e," had developed a germ tube and all of the others were forming conidia. This type of germination was relatively uncommon under the conditions used. (In one series 2 out of 30 spores gave rise directly to germ tubes, in two series most germinated in this fashion and in 12 series about half fused and the other half gave rise directly to germ tubes. In the remaining series hyphae seemed to arise in most cases from fused spores but in many cases it was impossible to be positive because of the crowded condition of the spores.) The variations in behavior noted may have been due to changes in the nutritive medium. Where mounts are held in a saturated atmosphere in moist chambers the moisture collecting on the slide and even dripping from the moist chamber to the slide at times would greatly vary the concentration of the liquid nutrient medium used.

When sporangiospores were planted on the surface of nutrient agar a wide spreading mycelium resulted exactly as when conidia were planted on agar. In all cases bacteria restricted the growth. Figures 68, 70, and 71 show germinating spores that were removed from the surface of agar (beef extract + dextrose + elm decoction) for examination under the microscope after 3 days' growth. In all three cases the hyphae seem to have come from fused germ tubes but it was impossible to be sure.

During germination it was often impossible to distinguish the "brims" of the hat-shaped spores but when observed the germ tubes usually seemed to emerge parallel with them (FIG. 62*i, d, c* ABOVE) while less often it emerged opposite the "brim" (FIG. 62*c* BELOW). While the "brims" were very difficult to see in unstained materials they show well in stained mounts, over a hundred of which have been examined.

The fusions of the germ tubes of sporangiospores resemble those well known for the ascospores of *Saccharomyces Ludwigi*, *Willia saturnus* and the yeast of Johannisberg II but the resemblance may be entirely superficial. No evidence has been secured to indicate that the fusion of germ tubes has a sexual significance.

During the process of germination the slime surrounding the

spores gradually disappears. Brefeld (1) observed this and concluded that it was used by the germinating spores. This is probably true but it does not seem to contain sufficient nutrition for their complete germination as in no case were well developed tubes secured unless additional nutrition was available. Brefeld's description of the germination of the sporangiospores quite markedly differs from that just described in certain details. He figures and describes the spores as rupturing in 2 halves much as is characteristic of the ascospores of *Aspergillus* and did not observe fusions of spores on germination. He, however, described the development of conidia from germinating sporangiospores. Holtermann (2) found that long, septate threads arise from the sporangiospores which gave rise neither to sporangia nor conidia. These are variations which might readily occur under varied cultural conditions.

#### CONCLUSIONS

1. An *Ascoidea*, held to be *A. rubescens*, was collected in slime fluxes at Ithaca, N. Y., and at Lincoln, Nebr. These findings apparently are the first records of the fungus in North America.
2. The development of sporangia and conidia, both from the slime flux and in cultures, has been studied.
3. Sporangia only develop on hyphae near the surface of water. A great reduction in the available nutrition is essential and alternate drying and wetting are favorable conditions.
4. Oval and cylindrical conidia develop either in the air or, if on submerged hyphae, near the surface. Thick-walled, resting conidia develop on submerged hyphae.
5. Exposure on the surface of agar or in thin films of liquid is essential for the germination of both conidia and sporangiospores.
6. Both types of spores upon germination give rise at once to conidia when nutrition is low and to well developed hyphae under conditions of higher nutrition. When very depleted conidia may almost directly give rise to sporangia upon germination.
7. Sporangiospores commonly fuse upon germination.

The writer wishes to express her thanks to Prof. H. M. Fitzpatrick, of Cornell University, for his stimulating interest in these

studies and for suggestions made after reading a draft of this paper to Prof. E. N. Andersen for assistance in making micro-chemical tests, and to Prof. T. J. Fitzpatrick, of the University of Nebraska, for editing and proof reading the paper.

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## NOTES ON SOME RUST COLLECTIONS FROM COLORADO, WYOMING, AND SOUTH DAKOTA<sup>1</sup>

H. W. THURSTON, JR., AND F. D. KERN

During the summer of 1929 Messrs. Fred J. Seaver and Paul F. Shope made some interesting collections of rusts in Colorado,<sup>2</sup> and, together with Mr. Junius Henderson, some further collections in South Dakota.

Their collections, together with several others which were made in Wyoming by the mycologists in attendance at the summer meeting of the Botanical Society of America, have recently been examined by the writers. In the list which follows, the collectors are not specifically named but should be understood to be as here indicated.

While the rusts of Colorado have become fairly well known, as the result of the work of such collectors as Arthur, Kern, Bethel, Gooding et al., the rust flora of Wyoming and South Dakota is not nearly so well known.

In determining these collections, several new localities were recorded for certain rust species as well as several hosts which are new, either for North America or for the region. In view of the fact that the volume on the Rusts of North America (Vol. 7 of the No. Am. Flora) is no longer open for the recording of additions and corrections in the distribution of rust species and their hosts it has seemed desirable to present a list of these recent collections. The list contains a total of ninety-seven collections, representing twelve genera and fifty-three species of rusts. Eleven host plants are here recorded as new for North America, one as new for Colorado, nine as new for Wyoming and seven as new for South Dakota, while six of the rust species are here reported from their respective localities for the first time. The three collections are numbered as one series; the numbers in this

<sup>1</sup> Contribution from the Department of Botany, the Pennsylvania State College, No. 73.

<sup>2</sup> See *Mycologia* 22: 1-8, 1930.

list are those as they appear on the specimens in the Herbaria of the New York Botanical Garden, the University of Colorado, and the Pennsylvania State College. The hosts were determined by P. A. Rydberg with the exception of those in the genus *Carex*, which were determined by K. K. MacKenzie.

**CALYPTOSPORA COLUMNARIS** (Alb. & Schw.) Kuhn on *Vaccinium* sp., Univ. of Wyoming Camp, Wyo., Aug. 1-4, 199.

**COLEOSPORIUM RIBICOLA** (Cooke & Ellis) Arth. on *Ribes inebrians* Lind., Gregory Canyon, Colo., Aug. 13, 349.

**CRONARTIUM HARKNESSII** (J. P. Moore) Meinecke on *Castilleja sulphurca* Rydb., Sylvan Lake, So. Dak., Aug. 8, 300. First report for South Dakota.

**GYMNOSPORANGIUM BETHELI** Kern on *Crataegus succulenta* Shrad., Gregory Canyon, Colo., Aug. 13, 348. New host for North America.

**MELAMPSORA BIGELOWII** Thüm. on *Salix* sp., Univ. of Colorado, Colo., July 29-30, 94.

**MELAMPSORA HUMBOLDTIANA** Speg. on *Salix amygdaloidea* Anderson, Edgemont, So. Dak., Aug. 6, 272. Wind Cave, So. Dak., Aug. 7, 277. New host for North America.

**MELAMPSOROPSIS PYROLAE** (DC.) Arth. on *Pyrola minor* L., Univ. of Wyoming Camp, Wyo., Aug. 1-4, 166. New host for Wyoming; the only previous report on this host is from Greenland.

**POLYTHELIS THALICTRI** (Chev.) Arth. on *Thalictrum dasycarpum* Fisch. & Lall., Wind Cave, So. Dak., Aug. 9, 329. Not before reported from South Dakota.

**PHRAGMIDIUM IVESIAE** Sydow on *Potentilla glaucophylla* Lehm., Univ. of Wyoming Camp, Wyo., Aug. 1-4, 208, 221. New host for Wyoming.

**PHRAGMIDIUM MONTIVAGUM** Arth. on *Rosa* sp., Univ. of Colorado Camp, Colo., July 29-30, 124. Gregory Canyon, Colo., Aug. 13, 362.

On *Rosa suffulta* Greene, Wind Cave, So. Dak., Aug. 7, 274. First report on this host for North America.

**PHRAGMIDIUM PECKIANUM** Arth. on *Oreobatus deliciosus* (Torr.) Rydb., Middle Boulder Canyon, Colo., July 23, 1.

**PHRAGMIDIUM SPECIOSUM** (Fries) Cooke on *Rosa* sp., Gregory Canyon, Colo., Aug. 13, 365.

- PUCCINIASTRUM AGRIMONIAE (Schw.) Tranz. on *Agrimonia striata* Michx., Legion Camp, So. Dak., Aug. 8, 316. New host for North America.
- PUCCINIA ABSINTHII (Hedw. f.) DC. on *Artemisia cana* Pursh.. Antelope, So. Dak., Aug. 6, 269, 270, near Edgemont, So. Dak., Aug. 10, 337. This is a new host for South Dakota. On *Artemisia cuneata* Rydb., Pikes Nat. Forest, Colo., Aug. 14, 378. New host for North America.
- PUCCINIA AEMULANS Sydow on *Gymnolomia multiflora* (Nutt.) B. & H., Pikes Nat. Forest, Colo., Aug. 16, 385.
- PUCCINIA ASTERUM (Schw.) Kern on *Aster* sp., Middle Boulder Canyon, Colo., July 26, 72. Univ. of Wyoming Camp, Wyo., Aug. 1-4, 214.
- PUCCINIA CIRSII Lasch. on *Cirsium megacephalum* (A. Gray) Cockerell, Gregory Canyon, Colo., Aug. 13, 353. On *Cirsium* sp., Univ. of Colorado Camp, Colo., July 29-30, 121.
- PUCCINIA CLEMATIDIS (DC.) Lagerh. on *Thalictrum* sp., Univ. of Colo. Camp, Colo., July 29, 123. On *Bromus ciliatus* L., Wind Cave, So. Dak., Aug. 9, 330. This is a new host for South Dakota.
- PUCCINIA CRUCIFERARUM Rudolphi on *Cardamine cordifolia* A. Gray, Univ. of Wyoming Camp, Wyo., Aug. 1-4, 161.
- PUCCINIA GENTIANAE (Strauss) Link on *Dasytephana* sp., Gregory Canyon, Colo., Aug. 13, 360.
- PUCCINIA GRINDELIAE Peck on *Grindelia texana* Scheele, Gregory Canyon, Colo., Aug. 13, 363.
- PUCCINIA GROSSULARIAE (Schum.) Lagerh. on *Ribes americanum* Mill., Wind Cave, So. Dak., Aug. 9, 331.
- PUCCINIA GRUMOSA Syd. & Holw. on *Anticlea coloradensis* Rydb., Middle Boulder Canyon, Colo., July 25, 70. First report for Colorado.
- PUCCINIA HEMISPHAERICA (Peck) E. & E. on *Lactuca pulchella* DC., Edgemont, So. Dak., Aug. 10, 339.
- PUCCINIA HELIANTHI-MOLLIS Schw. on *Helianthus lenticularis* Dougl., Univ. of Wyoming Camp, Wyo., Aug. 5, 265. New host for Wyoming. On *Helianthus fascicularis* Greene, Gregory Canyon, Colo., Aug. 13, 370.

PUCCINIA HEUCHERAE (Schw.) Dietel on *Mitella* sp., Univ. of Wyoming Camp, Wyo., Aug. 1-4, 256. This is a new locality for this rust. The specimen is referred to *P. Heucherae* although it differs somewhat since the spores are nearly colorless.

PUCCINIA HIERACIATA (Schw.) H. S. Jackson on *Agoseris parviflora* (Nutt.) Dietr., Univ. of Colorado Camp, Colo., July 29, 133.

On *Agoseris humilis* Rydb., Univ. of Wyoming Camp, Wyo., Aug. 1-4, 173. This is a new host for North America.

On *Agoseris scorzoneraefolia* (Schrad.) Greene, Univ. of Wyoming Camp, Wyo., Aug. 1-4, 211. This host is also new for North America.

On *Agoseris aurantiaca* (Hook.) Greene, Univ. of Wyoming Camp, Wyo., Aug. 1-4, 259. New host for Wyoming.

On *Agoseris* sp., Univ. of Wyoming Camp, Wyo., Aug. 1-4, 174, 261-II.

On *Carex siccata* Dewey, Univ. of Wyoming Camp, Wyo., Aug. 1-4, 212. New host for Wyoming.

PUCCINIA HIERACHII (Schum.) H. Mart. on *Taraxacum* sp., Univ. of Colorado Camp, Colo., July 29, 112.

On *Crepis* sp., Univ. of Colorado Camp, Colo., July 29, 122.

On *Taraxacum Taraxacum* Karst, Gregory Canyon, Colo., Aug. 13, 364.

On *Crepis runcinata* (James) T. & G., Wind Cave, So. Dak., Aug. 9, 296. New host for South Dakota.

On *Hieracium scabriusculum* Schw., Wind Cave, So. Dak., Aug. 7, 278. New host for United States.

PUCCINIA INTERMIXTA Peck on *Iva axillaris* Pursh, Edgemont, So. Dak., Aug. 6, 271. Edgemont, So. Dak., Aug. 10, 338.

PUCCINIA LIGUSTICI Ellis & Ev. on *Angelica Grayi* C. & R., Univ. of Wyoming Camp, Wyo., Aug. 1-4, 224. New host for Wyoming.

On *Ligusticum Porteri* Cooke & Rav., Univ. of Wyoming Camp, Wyo., Aug. 1-4, 216. New host for Wyoming.

On *Ligusticum simulans* Cooke & Rav., Univ. of Wyoming Camp, Wyo., Aug. 1-4, 169. New host for North America.

On *Oxypolis Fendleri* (Gray) Heller, Univ. of Wyoming Camp, Wyo., Aug. 1-4, 171, 176. New host for Wyoming.

- PUCCINIA MALVACEARUM Bertero on *Malva pusilla* Sm., Gregory Canyon, Colo., Aug. 12, 347. New host for North America.
- On *Althea rosea* Cav., Gregory Canyon, Colo., Aug. 13, 355.
- PUCCINIA MENTHAE Pers. on *Mentha Penardi* (Briq.) Rydb., Gregory Canyon, Colo., Aug. 13, 357. Also from Coal Creek Canyon, Colo., specimen not numbered.
- On *Monarda Ramaleyi* A. Nels., Wind Cave, So. Dak., Aug. 7, 273. New host for South Dakota.
- PUCCINIA POLYGONI-VIVIPARI H. Dietr. on *Bistorta vivipara* (L.) S. F. Gray, Univ. of Wyoming Camp, Wyo., Aug. 1-4, 175, 220.
- On *Oxypolis Fendleri* (Gray) Heller, I, Univ. of Wyoming Camp, Wyo., Aug. 1-4, 178. New host for North America.
- PUCCINIA POCULIFORMIS (Jacq.) Wettst. on *Hordeum jubatum* L., Edgemont, So. Dak., Aug. 10, 340. Gregory Canyon, Colo., Aug. 13, 372.
- On *Phleum pratense* L., Gregory Canyon, Colo., Aug. 13, 358.
- PUCCINIA SCABER (Ellis & Ev.) Barth. on *Stipa Lettermanii* Vasey, Pikes Nat. Forest, Colo., Aug. 16, 384.
- PUCCINIA SCANDICA Johans. on *Epilobium anagallidifolium* Lam., Univ. of Wyoming Camp, Wyo., Aug. 1-4, 177. New locality for this species, also new host for North America. The specimen bears also a few aecia of what appear to be *Puccinia Peckii* (Detoni) Kellerm.
- PUCCINIA SHERARDIANA Körn. on *Sphaeralcea coccinea* (Nutt.) Rydb., Gregory Canyon, Colo., Aug. 13, 368.
- PUCCINIA TRELEASIANA Paz. on *Caltha rotundifolia* (Huth.) Greene, Univ. of Wyoming Camp, Wyo., Aug. 1-4, 240. Middle Boulder Canyon, Colo., July 24, 38.
- PUCCINIA URTICATA (Link) Kern on *Carex nebrascensis* Dewey, Coal Creek Canyon, Colo., Aug. 23, 513.
- PUCCINIA VIOLAE (Schum.) DC. on *Viola adunca* Sm., Wind Cave, So. Dak., Aug. 7, 279. New host for South Dakota.
- PUCCINIA XANTHII Schw. on *Xanthium speciosum* Kearney, Antelope, So. Dak., Aug. 6, 268.
- UROPYXIS AMORPHAE (M. A. Curt.) Schröt. on *Amorpha canescens* Pursh., Wind Cave, So. Dak., Aug. 7, 280, Aug. 9, 334.
- UROPYXIS SANGUINEA (Peck) Arth. on *Odostemon aquifolium* (Pursh) Rydb., Gregory Canyon, Colo., Aug. 13, 352.

- UROMYCES AEMULUS Arth. on *Allium brevistylum* S. Wats., Univ. of Wyoming Camp, Wyo., Aug. 1-4, 162.
- UROMYCES ARGOPHYLLAE Seym. on *Psoralea argophylla* Pursh., Wind Cave, So. Dak., Aug. 7, 298, Aug. 9, 336.
- UROMYCES FABAE (Pers.) DeBary on *Lathyrus* sp., Pikes Nat. Forest, Colo., Aug. 14, 379.
- UROMYCES GLYCYRRHIZAE (Rab.) Magn. on *Glycyrrhiza lepidota* Nutt., Wind Cave, So. Dak., Aug. 9, 335.
- UROMYCES HETERODERMUS Sydow on *Erythronium parviflorum* (S. Wats.) Gooding, Univ. of Wyoming Camp, Wyo., Aug. 1-4, 163.
- UROMYCIES JONESII Peck on *Ranunculus alismellus* Geyer, Middle Boulder Canyon, Colo., July 26, 65. This is a new host for Colorado. Univ. of Wyoming Camp, Wyo., Aug. 1-4, 165, 209, 258. First report of this rust from Wyoming.
- UROMYCIES OBLONGUS Vize on *Trifolium Parryi* A. Gray, I, Univ. of Wyoming Camp, Wyo., Aug. 1-4, 219, 237. New host for Wyoming.  
On *Trifolium* sp., III, Univ. of Wyoming Camp, Wyo., Aug. 1-3, 223.
- UROMYCIES SILPHII (Burrill) Arth. on *Juncus Dudleyi* Wiegand<sup>4</sup> Gregory Canyon, Colo., Aug. 13, 359.
- UROMYCIES SUBSTRIATUS Sydow on *Lupinus argenteus* Pursh, Gregory Canyon, Colo., Aug. 13, 351.
- UROMYCIES TRIFOLII (Hedw. f.) Lév. on *Trifolium repens* L., Wind Cave, So. Dak., Aug. 8, 319. New host for South Dakota.

PENNSYLVANIA STATE COLLEGE,  
STATE COLLEGE, PA.

## A RARE PHALLOID FROM THE NEW YORK BOTANICAL GARDEN

FRED J. SEAVER

(WITH PLATE 8)

In 1916, David R. Sumstine reported a new species of *Colus* (*Mycologia* 8: 183. 1916) from Pennsylvania under the name *Colus Schellenbergiae*. The material from which this species was described was collected in the yard of Mrs. F. F. Schellenberg of Pittsburgh, Pennsylvania. The author of the species calls attention to the fact that the description of *Colus javanicus* Penzig agrees in a general way with his plants but states that he had no opportunity to compare the specimens.

In 1928, the writer discovered in an obscure corner of The New York Botanical Garden a group of phalloids which seem to agree in every way with the description given by Sumstine for his Pittsburgh material. This was collected throughout the entire summer, several of the plants appearing after each rain storm, the eggs remaining dormant during the intervening dry periods. Numerous collections were obtained and colored illustrations were made from the living material by Miss Mary E. Eaton. A report of this species was made before the Torrey Botanical Club at The New York Botanical Garden and a brief note published in connection with the minutes of that meeting (*Torreya* 29: 49. 1929). This has apparently been overlooked by mycologists, for no one has since mentioned the occurrence of this species outside of the Pittsburgh region.

Each season since the discovery of this plant in The New York Botanical Garden, it has continued to appear over an area scarcely more than twenty feet in diameter. Just why it should appear there and in no other place or how it got there are questions which it is impossible to answer. Since the above observations were made the writer has secured a small specimen of *Colus javanicus* from the Director of the Botanic Garden at Buitenzorg, Java. So far as it is possible to judge from preserved material

the American and Javan species are identical, as intimated by Sumstine in his original description.

During the winter of 1929, the writer received from Brother Leon of Cuba a specimen preserved in glycerine which also appears to be identical with the American and Javan specimens. This gives a very interesting distribution, assuming that the three are identical.

C. G. Lloyd has established a genus *Pseudocolus* to include those species of *Colus* in which the arms arise from a stem. This distinction would not appear to be a good one since in the young specimens the stem is not apparent, but appears as the specimens mature. The following is the synonymy of the species:

*COLUS SCHELLENBERGIAE* Sumstine, Mycologia **8**: 183. 1916.

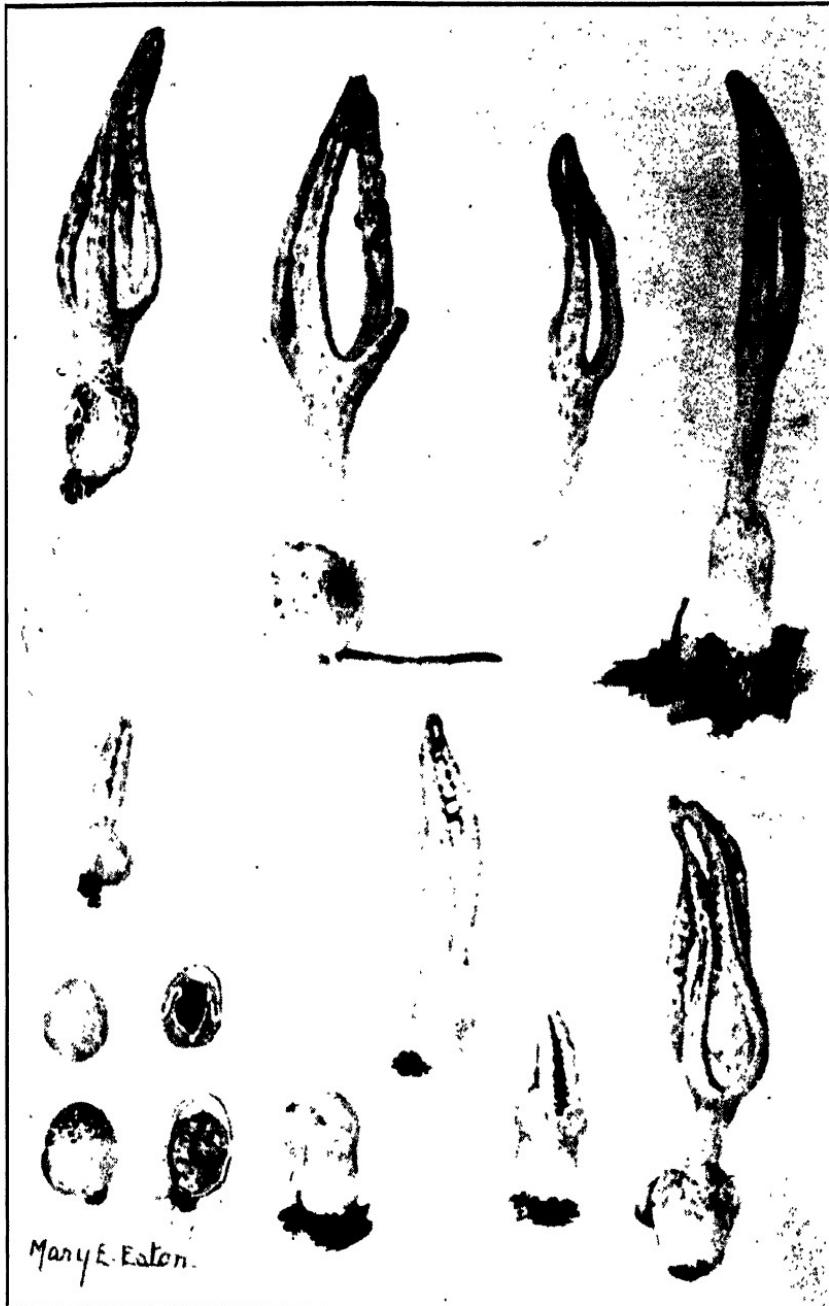
?*Colus javanicus* Penzig, Ann. Jard. Bot. Buitenzorg II. 1: 160. 1899.

*Pseudocolus Schellenbergiae* Johnson, Ohio Biol. Survey Bull. **22**: 338. 1929.

THE NEW YORK BOTANICAL GARDEN,  
BRONX PARK, NEW YORK

#### EXPLANATION OF PLATE 8

Photograph of the various stages in the development of *Colus Schellenbergiae* as it appeared in The New York Botanical Garden. The photographs were made from the colored drawings by Miss Mary E. Eaton and are about natural size.



COLUS SCHELLENBERGIAE



## NOTES AND BRIEF ARTICLES

While *MYCOLOGIA* is not usually sold separately, anticipating a demand for the present issue, which has been reproduced at rather heavy cost, extras have been printed and may be had through The New York Botanical Garden at one dollar (\$1.00) each.

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Doctor David W. Fairchild, long in charge of the office in the Bureau of Plant Industry dealing with plant introductions, visited the Garden Friday, October 31. Mycologists will be glad to learn that he is still interested in *Basidiobolus*, a fungus on the cytology of which he made an important contribution many years ago. Now with every opportunity and condition favorable to research may we not hope to see Dr. Fairchild endeavor to clear up some of the puzzling questions still being raised regarding the nature of sex and reproduction in such fungi.

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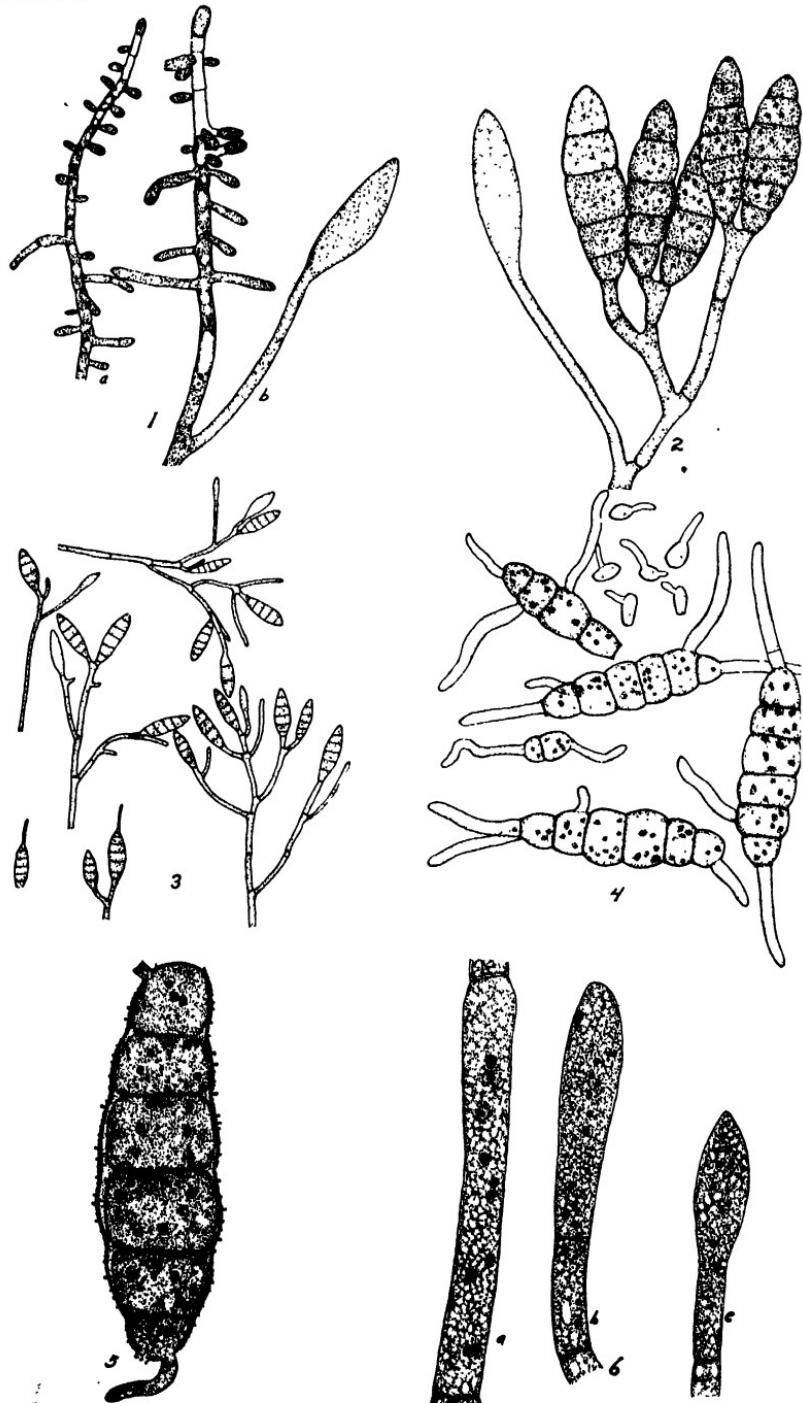
Dr. Alfred H. Povah is spending this year at the Cryptogamic Laboratories of Harvard University assisting Professor William H. Weston, Jr., and in the Farlow Herbarium studying the fungi collected last summer by the Botanical Party of the Michigan State Survey of Isle Royale, Lake Superior, under the auspices of the University of Michigan Herbarium. Approximately 2100 collections of fungi and lichens were obtained by the party consisting of Dr. Alfred H. Povah, in charge of Cryptogams, assisted by Mr. J. L. Lowe, Jr., and Mr. Clair A. Brown, in charge of Phanerogams, assisted by Mr. J. B. McFarlin.

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The New York Botanical Garden has recently received a valuable addition to its collection of cultures of fungi from Dr. Charles Thom of the Bureau of Chemistry and Soils, Department of Agriculture, Washington, D. C. This gift, consisting of something over two hundred cultures of species of *Aspergillus*

and *Penicillium*, is especially valuable at this time, since the Garden is cooperating with the Laboratory of Mycology, Department of Dermatology, of the College of Physicians and Surgeons of Columbia University, and it is known that several species of the fungi in these groups have been found associated with certain cases of asthma and other human diseases. The set is one of five being distributed to central laboratories in the United States. We are certainly very grateful to Dr. Thom for his contribution.





ACHORION GYPSEUM

# MYCOLOGIA

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No. 2

## OBSERVATIONS ON ACHORION GYPSEUM

C. W. EMMONS

(WITH PLATES 9 AND 10 AND 1 TEXT FIGURE)

*Achorion gypseum* Bodin is a pathogenic fungus which reproduces in culture by means of small single-celled conidia and large pluriseptate macroconidia, commonly called aleuries and fuseaux respectively by medical mycologists. Three cultures of this fungus were received from the laboratory of Dr. Sabouraud. One of these three strains, designated in our collection of cultures of human pathogens as No. 7, has been used in an attempt to learn whether cultures which grow from the small conidia differ from those which grow from the macroconidia or fuseaux. Ordinarily, in the transfer of cultures a mixture of these two types is used. *A. gypseum* is favorable for this study because on suitable media it produces both types of spores simultaneously and in large numbers. It is of further interest because it has been reported that it forms asci when grown on suitable substrates of animal origin such as leather and feathers.<sup>1</sup>

The fungus was named by Bodin,<sup>2</sup> who in 1907 isolated it from a typical case of favus. The patient was a woman thirty years of age who had on the right cheek an erythematous-

<sup>1</sup> Nannizzi, A. Ricerche sull'origine saprofytica dei funghi delle tigne. Il *Gymnoascus gypseum* sp. n. forma ascofera del *Sabouraudites (Achorion) gypseum* (Bodin) Ota et Langeron. (Nota preventiva.) Atti Accad. Fisiocritici Siena. X. 2: 89-97. 1927. (Abstract in Rev. Appl. Myc. 7: 169. 1928.)

<sup>2</sup> Bodin, E. Sur un nouveau champignon du favus (*Achorion gypseum*). Ann. Dermat. & Syph. 8: 585-602. 1907.

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squamous lesion with four typical favus godets. Sabouraud<sup>3</sup> believes that this is the same fungus which he saw in 1894 and described briefly in his book "Trichophytes humaines" as the cause of a benign kerion. The fungus is of animal origin and has been isolated from the horse.<sup>4</sup> It resembles species of *Microsporum* of animal origin. Sabouraud finds that the fungus is capable of infecting the glabrous skin, the beard, or the scalp. Examination of the squames shows an abundant mycelium, and uninfected hairs are more numerous than infected ones. Of those infected, some hairs are invaded while others are surrounded by a sheath but are not invaded.

There is a close resemblance between *Achorion gypseum* and *Microsporum fulvum* and the two appear to be closely related. Ota and Langeron<sup>5</sup> place both of them in the genus *Sabouraudites*. Both produce some conidia and many macroconidia, and in old cultures the latter occur in great abundance. In *A. gypseum* the two types of spores are borne on different parts of the mycelium (PLATE 9, FIGS. 1a, 2), or they arise in close proximity (PLATE 9, FIG. 1b).

It seemed possible that the downy or "pleomorphic" type of culture which develops in old cultures might arise prevailingly from only one of the spore types which are present in this fungus. In a paper in which he puts forward a reclassification of the dermatophytes and records valuable cytological data for many forms including *A. gypseum*, Grigorakis<sup>6</sup> presents this hypothesis. He considers the term pleomorphism inappropriate and believes that the dermatophytes exhibit neither polymorphism nor pleomorphism but a monomorphism in which there is a progressive degeneration of both mycelial and fruiting structures. He believes the conidia are different in nature from the fuseaux and represent the degraded forms of the latter developed during this progressive degeneration. Observations made on mono-

<sup>3</sup> Sabouraud, R. Les Teignes, 571-591, 709-713. 1910.

<sup>4</sup> Urbain, Barotte, & Capdebielle. Sur un cas de teigne équine due à l'*Achorion gypseum*. Bull. Mém. Soc. Centr. Méd. Vétér. 102: 50. 1926. (Abstract in Rev. Appl. Myc. 5: 555. 1926.)

<sup>5</sup> Ota, M. & Langeron, M. Nouvelle classification des dermatophytes. Ann. Parasitol. 1: 305-336. 1923.

<sup>6</sup> Grigorakis, L. Dermatophytes et dermatomycoses. Ann. Dermat. & Syph. 10: 18-68. 1929.

spore cultures would have given evidence bearing directly on the genetic nature of the different types of spores he studied, but he does not record such data.

The term pleomorphism was originally used in mycology to describe the supposed variability of fungi. Distinct species of fungi which often follow each other in impure cultures were at first thought to be different forms of a single species. Pure culture methods showed that *Mucor* and *Aspergillus*, for example, are not forms of the same organism. The words pleomorphism and, more commonly, polymorphism are still occasionally used by some mycologists to describe either the presence of two or more spore forms in a life cycle or the variability of a species.

Medical mycologists, on the other hand, have given to the term pleomorphism a special meaning. Sabouraud, when he first studied the overgrowth of white downy mycelium on his cultures, believed that he was observing a case of commensalism. He (l.c., p. 84) credits Bodin with being the first to show that this was not a correct interpretation. Bodin<sup>7</sup> believed that it was a case of "polymorphisme" such as had been demonstrated for many of the saprophytic fungi. Later he and Sabouraud together studied this phenomenon and distinguished between the words polymorphism and pleomorphism. Fox and Blaxall<sup>8</sup> seem to have been first to apply the term pleomorphism to this phenomenon although they apparently did not differentiate between pleomorphism and polymorphism. Sabouraud defines pleomorphism of the dermatophytes as a stable variation involving degeneration and loss of complexity and giving rise to a new form which can not revert to the original type. This view, from which that of Grigorakis differs fundamentally, is accepted by nearly all dermatologists. This conception is clearly stated by Brumpt,<sup>9</sup> who says: "Le polymorphisme est donc un simple phénomène d'adaptation qui ne doit pas être confondu avec le pléomorphisme. . . . Pour Sabouraud . . . le pléomorphisme des

<sup>7</sup> Bodin, E. Les teignes tondantes du cheval et leurs inoculations humaines. Thèse de Paris. 1895.

<sup>8</sup> Fox, T. C. & Blaxall, F. R. An inquiry into the plurality of fungi causing ringworm in human beings as met with in London. Brit. Jour. Dermat. 8: 340. 1896.

<sup>9</sup> Brumpt, E. Précis de Parasitologie, 1147. 1927.

*Champignons des teignes constitue une véritable variation stable, donnant naissance à des formes nouvelles cultivables et inoculables, incapables de revenir au type primitif même après passage sur l'animal et quand des conditions biologiques différentes lui sont offertes.*" The nature of this change is important to an understanding of the dermatophytes and their relations to their hosts. Sabouraud<sup>10</sup> in a recent article has called this change a fixed mutation. Whether or not it is comparable to the so-called mutations or saltations so frequently described in other fungi by mycologists is a question. A study of monospore cultures clearly shows that the two types of spores simultaneously produced by *A. gypseum* are of equal rank, and that the "pleomorphic" change is not due to the development of cultures from only one of these spore types.

It is obvious that monospore culture methods are important in these studies of variation in human pathogens as well as in the determination of heterothallism where they have yielded such brilliant results. Many methods and many mechanical devices have been employed in the isolation of single spores from which cultures can be secured. For very minute spores special spore pickers are useful, especially in the hands of an expert. However in most cases no apparatus is required other than a dissecting microscope and a very fine needle honed down to a cutting chisel-like edge. The method described below is in common use by many mycologists. A suspension of the spores of the fungus is made in sterile distilled water or saline. This suspension is poured or sprinkled over the surface of a plate of four per cent cornmeal agar or other hard clear agar. The liquid is then evenly spread over the surface of the plate by tilting it or by streaking with a loop, and it is soon absorbed by the hard agar. The suspension must be sufficiently heavy so that the spores are well separated without being too close together to be picked out later, or so far apart as to be difficult to find. The Petri dish is now set aside until the spores germinate. In the case of *A. gypseum* this will be within 10 hours (TEXT

<sup>10</sup> Sabouraud, R. Généralités concernant les dermatophytes. Le problème du pléomorphisme des cultures des dermatophytes. V<sup>e</sup> Mémoire. Ann. Dermat. & Syph. 10: 481-486. 1929.

FIG. 4). Germination begins within 3 or 4 hours, but if left for 8 or 10 hours practically all the viable spores have begun to germinate and it is then possible to choose spores which are certainly isolated. The plate is uncovered and placed on the stage of a dissecting microscope with an objective of moderate magnification. The microscope stage and table top should be wetted down with lysol, and subsequent operations must take place in a room with windows closed and free from floating dust



FIG. 1. Germinating macroconidia after ten hours.

and spores. A spore well isolated is chosen and is then cut out with a very fine steel needle while under observation through the microscope. It is then picked up and transferred to a tube or plate. Either the entire germinated spore or a free hyphal tip from it can be transferred in this manner. It is convenient to dip the needle between transfers into a vial of alcohol and flame quickly in order to avoid burning away the fine needle point. After a little practice this method can be readily employed, and under ideal conditions single spore cultures can be made easily,

quickly, and with confidence that not more than one spore was used as the inoculum.

Single spore cultures were made using both the small conidia and the macroconidia. The two types of cultures gave nearly identical pictures (PLATE 10). The only observable difference was in the gross appearance of the colonies. Those from the macroconidia were in nearly all cases slightly larger than the others and appeared to be 24-48 hours in advance of the other type. This is easily explained when one examines the germinating spores. The macroconidia germinate before the conidia and produce much larger germ tubes. The mycelial network arising from such a spore receives a more vigorous start (PLATE 9, FIG. 4). This is perhaps due to the greater amount of food in the spore. Plate 10, figure 7, shows a photograph of a plate culture on Sabouraud's maltose agar medium, the culture originating from a single conidium of *A. gypseum*. Figure 10 is of a culture of the same age and on the same medium which developed from a single macroconidium. Microscopically the cultures are identical. There is the same proportionate occurrence of the spore forms in both, and subcultures from the two cultures give identical pictures both macroscopically and microscopically. Figures 8 and 11 are of other plate cultures, and figures 9 and 12 are of tube cultures corresponding in a similar manner. Both types of cultures become covered by the "pleomorphic" overgrowth of mycelium at about the same time. This pleomorphic mycelium produces very many conidia and a few small two- to four-celled macroconidia. Monospore cultures from these two types of spores are alike in every way. The cultures are pleomorphic, differing from the parent culture by lack of pigmentation, more abundant aerial mycelium, and relatively larger numbers of the small conidia. Some change has occurred in the fungus, but it is a change which has affected both types of spores. The spores which are simultaneously produced on the mycelium appear still to be identical in their nature.

Many other cultures from single conidia and macroconidia were studied in other series, and the results were always the same. I find also that in an extended series of cultures derived from single conidia and macroconidia of a fungus of the *Trichophyton*

*gypseum* group the two kinds of spores give identical cultures. There is no evidence that the two types of spores which are formed simultaneously are genetically different. The conidia formed in pleomorphic cultures do, however, differ from those formed in the original type of culture. Just how and where this change takes place is not yet clear.

For a study of the nuclear condition colonies of the fungus growing on agar were fixed, sectioned, and stained. Young colonies should be chosen, and there is some advantage in using such a culture medium as cornmeal agar<sup>11</sup> which often stimulates spore production but does not favor the development of an extensive cottony mycelium. Several different fixatives were tried. Best fixation was secured with Flemming's weaker fluid allowed to act for one hour.

The cells of *A. gypseum* are comparatively long and are multinucleate as is shown in plate 9, figure 6a. This fact has also been noted and figured by Grigorakis. The presence of as many as 18 nuclei in one cell has been noted although there are not usually so many. These nuclei are variable in their arrangement and position. They may be paired in the cell as though two newly formed daughter nuclei had not yet separated, or they may be scattered. In some cases the nuclei are near one end while in others they are distributed throughout the length of the cell. The young macroconidia are also multinucleate (PLATE 9, FIGS. 6b, c). At germination the cells of these spores contain several nuclei (PLATE 9, FIG. 5). As many as ten may be present. The conidia are usually uninucleate but may contain two nuclei. Each nucleus contains one large nucleole. I was unable to observe a chromatic reticulum, and the nuclear membrane is very difficult to make out in many nuclei.

Cultivation of this and other pathogenic fungi on various organic substrates such as silk, wool, feathers, litter, and wood has been reported.<sup>12</sup> I have cultivated *A. gypseum* on a variety

<sup>11</sup> To 3 liters of distilled water add 125 g. cornmeal (water ground); heat in water bath 1 hr. at 60° C.; filter through filter paper; make up to 3000 cc.; add 37.5 g. agar (1½%); steam for 1¼ hrs.; filter through absorbent cotton; flask and sterilize in autoclave at 15 lbs. pressure 30 min.

<sup>12</sup> Kadisch, E. T. Über das Fortkommen der pathogenen Hautpilze ausserhalb des Körpers. Dermat. Wochenschr. 89: 1423-1433. 1929.

of media with the hope of securing other fruiting forms. It grows well on sterilized human skin, nail parings, horn, wool, feathers, and on agar to which the only nutrient added was finely divided horn, and it liquefies gelatine; but on all of these it has so far produced only its usual spore forms. It does however occasionally produce spirals when grown on nail parings. Langeron and Milochevitch<sup>13</sup> report the presence of spirals in *A. gypseum* when it is grown on various grains. Nail parings sterilized in tubes in the bottoms of which have been placed moistened wads of cotton are, after inoculation with *A. gypseum*, quickly overgrown and the substance of the nail is destroyed. Horn is equally suitable as a culture medium.

Summary: Conidia and macroconidia (fuseaux) of *Achorion gypseum* are not genetically different. The hyphal cells and the macroconidia are multinucleate; the small conidia have one or sometimes two nuclei. Spirals such as those which are considered characteristic for *Trichophyton asteroides* are sometimes produced when the fungus is grown on nail parings. It grows well on nail parings and on pieces of horn and destroys the substrate.

Dr. R. A. Harper, Dr. J. G. Hopkins, and Dr. B. O. Dodge have offered helpful criticisms during the preparation of this paper.

FROM THE LABORATORY OF MEDICAL MYCOLOGY,  
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COLUMBIA UNIVERSITY

#### EXPLANATIONS OF PLATES

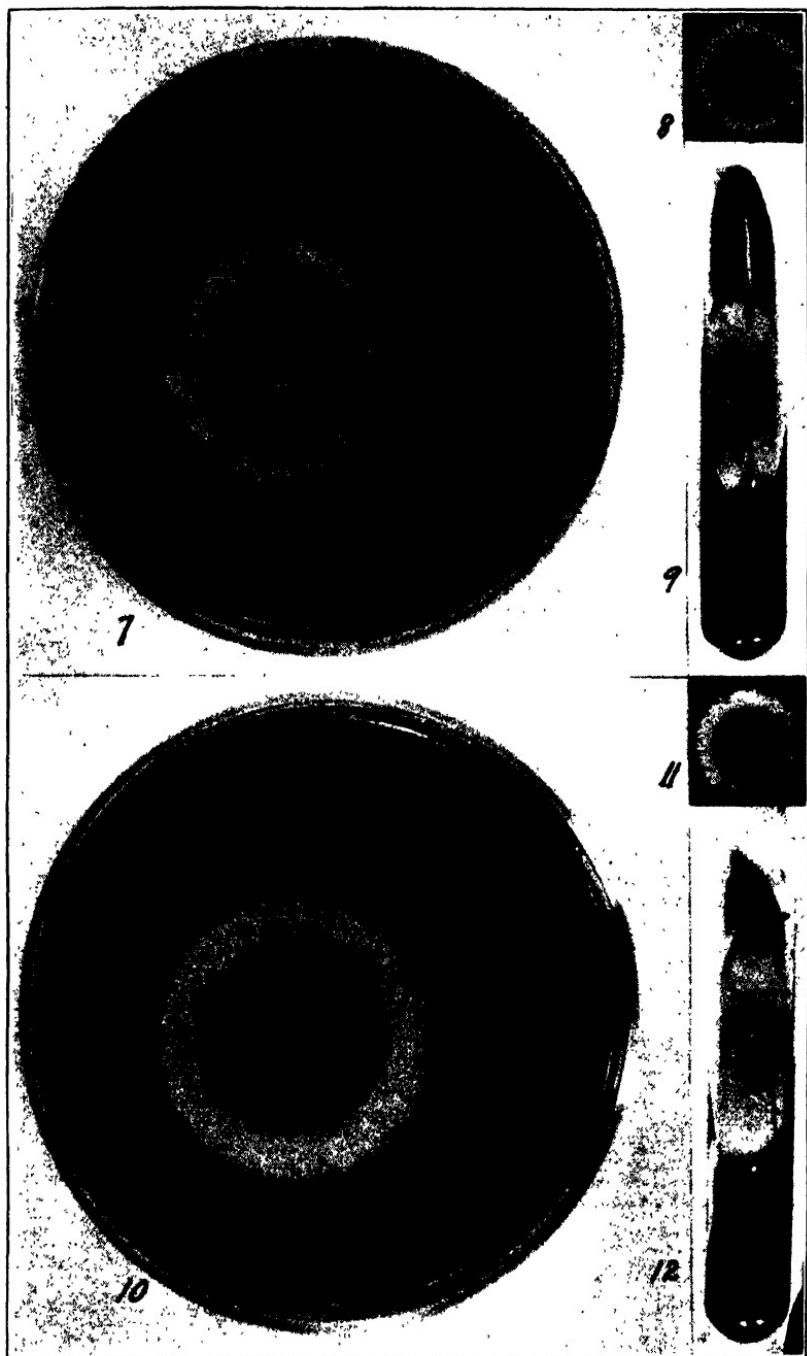
##### PLATE 9

Fig. 1. Mycelial branches bearing spores: *a*, a branch bearing conidia only; *b*, conidia and macroconidia produced on adjacent branches of the mycelium.  $\times 470$ .

Fig. 2. A cluster of macroconidia showing spores of different ages.  $\times 470$ .

Fig. 3. Typical groups of macroconidia. Cornmeal agar culture. Some of the spores have germinated by sending out a germ tube from the free end.  $\times 100$ .

<sup>13</sup> Langeron, M. & Milochevitch, S. Morphologie des dermatophytes sur milieux naturels et milieux à base de polysaccharides. Ann. Parasitologie 8: 422-436. 1930.



ACHORION GYPSEUM



Fig. 4. Germinating conidia and macroconidia after five hours on agar at room temperature.  $\times 470$ .

Fig. 5. Germinating macroconidium stained.  $\times 1300$ .

Fig. 6. A vegetative hypha and two young macroconidia stained to show nuclei.  $\times 1300$ .

#### PLATE 10

Fig. 7. Plate culture on Sabouraud's maltose agar medium of a monospore culture of *Achorion gypseum* derived from a single conidium.

Fig. 8. Same as Fig. 7.

Fig. 9. Tube culture from a single conidium.

Fig. 10. Plate culture on Sabouraud's maltose agar medium of a monospore culture derived from a single macroconidium.

Fig. 11. Same as Fig. 10.

Fig. 12. Tube culture from a single macroconidium.

NOTE. While this paper was in press a strain of *Achorion gypseum* has been isolated in this laboratory by Miss R. W. Benham. It was recovered from a white boy five years of age with a kerion of the scalp. There were no lesions suggesting favus. This is apparently the first instance of the observation of *A. gypseum* or the probably identical species, *Microsporum fulvrum*, in this country. Bloch, in his *Handbuch der Haut- und Geschlechtskrankheiten*, Vol. 11, page 81, states that it was reported from New York by Mewborn in 1903. Mewborn in volume 21, pages 11-18, of the *Journal of Cutaneous Diseases* for 1903 does describe a fungus which he isolated from ringworm and favus-like lesions on the same patient. An examination of his description and photographs of cultures however make it appear that he did not have the fungus which Bodin isolated and described four years later.

# THE RUSTS OF SOUTH AMERICA BASED ON THE HOLWAY COLLECTIONS—III<sup>1</sup>

H. S. JACKSON

(WITH PLATE 11 AND 5 TEXT FIGURES)

The present contribution is the third of a series<sup>2</sup> bearing the same title. This number contains a continuation of the records of the rusts on Berberidaceae and on other host families in the order given in Engler and Prantl through the Rosaceae.

In order to facilitate indexing each species is given a number and the numbering is consecutive for the three papers. Including those recorded in this paper, a total of 99 species and 220 collections are now accounted for. In a few cases reference is made to species not included in the Holway collections. Where such references are of an important character a duplicate number has been given with the suffix *a*, *b*, etc., in order to keep the numbering of the species in the Holway collections consecutive.

## SPECIES ON BERBERIDACEAE

(continued)

### 83. *Puccinia Berberidis-Darwinii* Jackson & Holway, nom. nov.

*Caeoma Berberidis* Diet. & Neger; Engl. Bot. Jahrb. 27: 13. 1899. Not *C. Berberidis* (Lév.) Har. 1891, nor *P. Berberidis* Mont. 1856.

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*Berberis Darwinii* Hook. f. Lago Llanquihue, Chile, Dec. 2, 1919, I, III. 196; Puella, Lago Todas los Santos, Chile, Nov. 30, 1919, I. 193.

This interesting species was first described as *Caeoma Berberidis* Diet. & Neger. It has apparently not often been collected, as the only previous record seems to have been that for the type collection which was made on the same host in Chile, by Neger.

<sup>1</sup> Joint contribution from the Department of Botany, Purdue University Agricultural Experiment Station, and the Department of Botany, University of Toronto.

<sup>2</sup> See *Mycologia* 18: 139–162. 1926; 19: 51–65. 1927.

Our specimens do not show any amount of hypertrophy in association with aecial infection but the sori occur singly or in small groups on the underside of the leaves.

Pycnia have not been described for this species but are abundant in collection 193. They are either epiphyllous or hypophyllous but more commonly epiphyllous, obovate or globoid, deep seated, 100–125 by 110–150  $\mu$ . Telia occur in collection 196. These are hypophyllous, scattered and subepidermal. Aeciospores (?) occur in many of these sori. These are in short chains as in the aecia. Such sori are apparently unaccompanied by pycnia. The teliospores are clavate, rounded above and narrowed below, 16–20 by 55–80  $\mu$ . The upper cell varies in length from  $\frac{1}{2}$  to  $\frac{3}{4}$  that of the lower cell. The wall is  $1\frac{1}{2}$ – $2\frac{1}{2}$   $\mu$  in thickness, with the apex gradually thickened, 3– $3\frac{1}{2}$   $\mu$ . The pedicel is colorless, persistent, equaling the spore in length or longer. The pore in the upper cell is apical.

This very interesting species raises some important questions as to its systematic position. The aecia are not aecidium-like but resemble those of *Gymnoconia* or *Phragmidium*. The pycnia are, however, sub-epidermal. The occurrence of spores identical with the aeciospores in the telial sori suggests that this species possesses repeating aecia as in *Puccinia Senecionis*, *Uromyces Hedyosari-obscuri*, etc., but with quite different gross morphology.

These characters might justify the erection of a new genus to accommodate this species but we feel that until a detailed cultural study is available, and until a more detailed morphological study has been made than is possible with the relatively meager material at our disposal, it is preferable to include it in *Puccinia*.

#### **Edythea Jackson, gen. nov.**

Uredinia and telia superficial, formed at the apex of erect hyphae which emerge singly or in groups of two or three through the stomata of the host. Urediniospores and teliospores stipitate, originating from cells formed as the result of a close, short, irregular branching at or near the apex of the emerged hyphae. Teliospores two-celled with a single germ pore in each cell.

Type species. *Uropyxis quitensis* Lagerh. 1918.

This genus is erected to accommodate three species of rusts occurring on *Berberis* in South America. A careful study has revealed that both urediniospores and teliospores are borne in a very characteristic and presumably unusual manner. There is no sorus in the usual sense of the term. Mycelial threads emerge singly or in groups of two or three, rarely perhaps more, from the stomata. The stomata are not ruptured. There is no appreciable fungus tissue in the substomatal cavity. It appears that some of the mycelium threads which reach this cavity merely

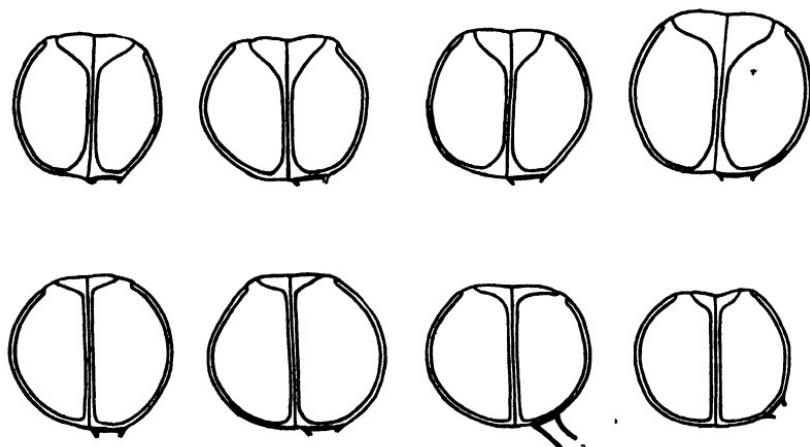


FIG. 1. *Edythea quitensis*. Spores from Holway collections 980 and 918.

turn outward through the stomatal opening and, having emerged, form a close system of short branches from the ultimate cells of which the urediniospores or teliospores arise on short colorless pedicels (FIGURES 4 AND 5). The gross appearance is that of an olive-green or cinnamon-brown hyphomycete. In all three species the teliospores germinate as soon as formed by the development of a four-celled basidium. The urediniospores in the three species are much alike. The teliospores are, however, quite different. The following key will serve to separate the species.

- Teliospores as in *Sphenospora*, with vertical septum.. 84a. *E. Berberidis*.  
Teliospores as in *Puccinia*, septum variable.  
    Teliospores nearly globoid ..... 84. *E. quitensis*.  
    Teliospores ellipsoid ..... 85. *E. tenella*.

84. *Edythea quitensis* (Lagerh.) Jackson & Holway, comb. nov.

*Uropyxis quitensis* Lagerh.; Arth. Bot. Gaz. 65: 464. 1918.  
*Sphenospora quitensis* Lagerh. in herb. Arth.

*Berberis phyllacantha* Rusby. Sorata, Bolivia, Apr. 27, 1920.  
 579.

*Berberis* sp. Quito, Ecuador, August 18, 1920. 918;  
 Cuenca, Ecuador, September 12, 1920. 980.

This species is based on a specimen in the Arthur herbarium collected by G. Lagerheim at Quito, Ecuador, in April 1891. This specimen is labeled *Sphenospora quitensis* Lagerh. n. sp. It was first described by Arthur in 1918 as *Uropyxis quitensis* Lagerh. The three collections made by the Holways agree well with the type. In this species the spores are nearly spherical, about as long as broad, with the pedicel variously attached with reference to the septum (FIG. 1).

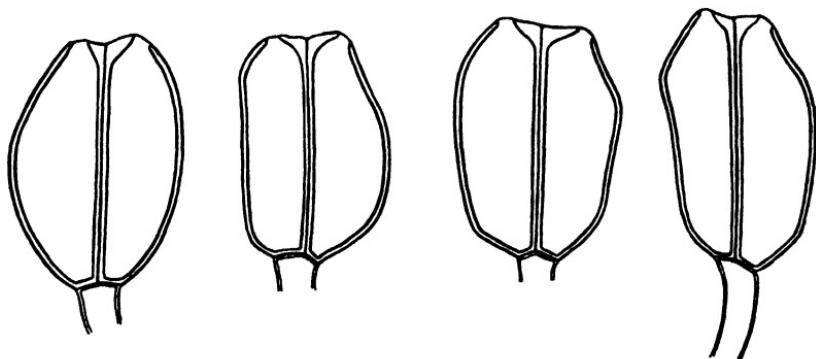


FIG. 2. *Edythea Berberidis*. Spores from type collection.

84a. *Edythea Berberidis* (Lagerh.) Jackson, comb. nov.

*Sphenospora Berberidis* Lagerh.; Arth. Bot. Gaz. 65: 464. 1918.

*Diorchidium Berberidis* Lagerh. in herb. Arth.

This species is known from a single collection made on *Berberis glaucescens* St. Hil. at Tahatanga, Ecuador, in September 1891 by G. Lagerheim. The type specimen was labeled *Diorchidium Berberidis* Lagerh. n. sp. In gross features this species has the

same habit as *Edythea quitensis*. The teliospores are, however, quite different in that the spores are much narrower in proportion to their length and the septum is quite uniformly vertical, the pedicel being attached at or near the septum (FIG. 2). This species was not collected by the Holways but is inserted here as its relationship is clearly with this genus.

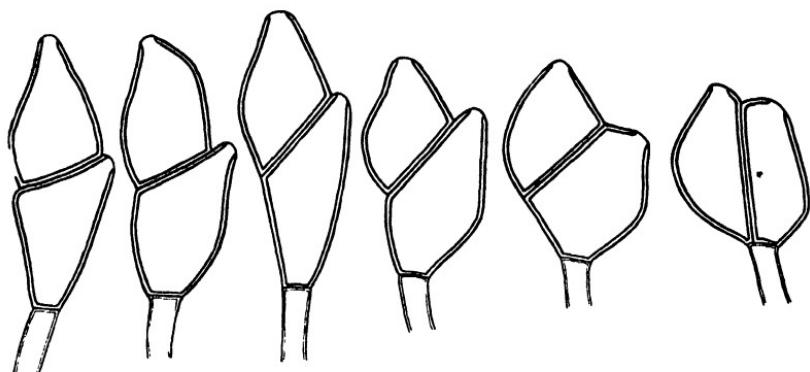


FIG. 3. *Edythea tenella*. Spores from type collection.

85. ***Edythea tenella*** Jackson & Holway, sp. nov. (TEXT FIGURES 3, 4 AND 5.)

II. Uredinia hypophylloous, superficial and effused, occurring in scattered greyish patches  $\frac{1}{2}$ -2 mm. across; urediniospores borne on basal cells originating from the close branching of threads which emerge singly or in groups of 2 or 3 from the stomata, globoid,  $23-25 \mu$  in diam.; wall thin,  $1-1\frac{1}{2} \mu$ , evenly, prominently and rather closely echinulate; pores obscure.

III. Telia like the uredinia, cinnamon-brown; teliospores variable, ellipsoid or fusiform, 16-20 by 30-50  $\mu$ , rounded or slightly narrowed above and below, constricted at the septum, germinating at once; wall light golden brown,  $1 \mu$  in thickness, slightly thickened about the germ pores which are located in the apex in the upper and near the septum in the lower cell; pedicel short, colorless.

*Berberis divaricata* Rusby. Sorata, Bolivia, April 22, 1920.  
564 (type).

This species differs from the other two in the character of the teliospores. The majority of these are ellipsoid and not unlike many species of lepto-*Puccinia*. Many of them, however, are

quite variable in shape due to the variable position of the septum. In some spores the septum is oblique and the walls round out giving a characteristic appearance. In others the septum becomes nearly vertical, approaching the condition in *E. Berberidis* (FIG. 3). This type collection has an *Aecidium* on the fruits but we hesitate to suggest that they belong with the uredinia and telia.



FIG. 4. *Edythea tenella*. Photograph from type collection showing cluster of spores originating from a single hypha which has emerged through a stomate.

86. PUCCINIA MONTANENSIS Ellis, Jour. Myc. 7: 274. 1893.

*Dicaeoma montanensis* Kuntze, Rev. Gen. 33: 469. 1898.

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*Berberis* sp. Termas de Chillán, Chile, Dec. 28, 1919, I. 254.

This *Aecidium* on *Berberis* was assigned, as indicated above, by Arthur in his contribution to the grass rusts of South America

(Proc. Am. Phil. Soc. **44**: 167. 1925). It is included here for the sake of completeness. Uredinia and telia occur in South America on several species of *Bromus* and *Elymus*.

#### SPECIES ON LAURACEAE

##### 87. *Aecidium Nectandrae* Jackson & Holway, sp. nov.

O. Pycnia epiphyllous, numerous, scattered on discolored spots, globoid, very large, 170–210  $\mu$ , deep seated, arising from below the palisade layer, finally rupturing the epidermis with ostiole as broad as the diameter of the pycnium.

I. Aecia hypophyllous, numerous, scattered on discolored spots 1–3 cm. across; peridium short, cylindric, firm, erose at margin; peridial cells rhombic in cross section, 18–20  $\mu$  long by 15–20  $\mu$  high, outer wall 1.5–2.5  $\mu$ , smooth, inner wall 2–3  $\mu$ , roughly tuberculate; aeciospores ellipsoid or short cylindric, 16–20 by 23–28  $\mu$ ; wall finely and prominently verrucose, the apex thickened, 3.5–5.5  $\mu$ .

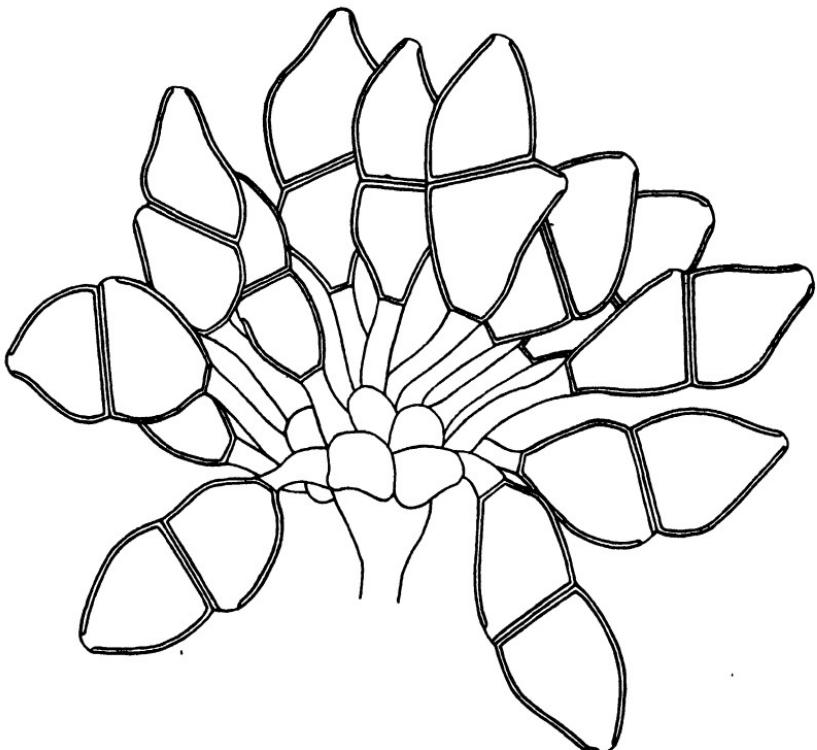


FIG. 5. *Edythea tenella*. Drawing from type collection showing cluster of spores originating from a single hypha which has emerged through a stoma.

*Nectandra oppositifolia* Ness. Bello Horizonte, Minas Geraes. Brazil, November 26, 1921. 1339.

This appears to be a very distinct species occurring on large spots which sometimes coalesce covering considerable areas. The pycnia are very large and when mature have a spreading ostiolum appearing in cross-section to be short cylindric with a rounded base deeply seated in the tissues of the host.

#### SPECIES ON CAPPARIDACEAE

##### 88. *Puccinia Cleomis* Jackson & Holway, sp. nov.

O. Pycnia not seen, probably not present.

III. Telia hypophylloous, small, 0.2–0.5 mm. across, closely gregarious in small group 2–3 mm. across on discolored spots, early naked, pulvinate, firm, ruptured epidermis not conspicuous; teliospores broadly ellipsoid or clavate, 19–24 by 35–44  $\mu$ , apex rounded, base rounded or somewhat tapering; wall thin, 1.5–2.5  $\mu$ , broadly thickened at apex, 3–4  $\mu$ , smooth; pedicel colorless, firm, equaling the spore or shorter, occasionally longer.

*Cleome gigantea* L. San Felipe, Sur Yungas, Bolivia, May 21, 1920. 634.

#### SPECIES ON SAXIFRAGACEAE

##### 89. *Uromyces ribicola* Jackson & Holway, sp. nov.

II. Uredinia hypophylloous, numerous, scattered, small, round, 0.2–0.5 mm. in diameter, golden-brown; urediniospores globoid or broadly ellipsoid; wall colorless, 2–3  $\mu$  in thickness below, irregularly and greatly thickened, 10–13  $\mu$  above, evenly and moderately echinulate; pores obscure, several.

III. Telia like the uredinia, whitish, ruptured epidermis not conspicuous; teliospores cylindrical or occasionally bent to one side near apex, 16–19 by 75–115  $\mu$ ; wall colorless, very thin, 1  $\mu$  or less, not thickened at apex, no germ pore evident, germinating at once.

*Ribes albiflorum* R. & P. Sorata, Bolivia, Apr. 22, 1920. 568.

This species is assigned to *Uromyces* with considerable doubt. The teliospores are thin-walled and uninterrupted in development. It appears that in the development of teliospores there is no cessation of growth till the basidium is fully formed and there

is no point at which one may say that the spore ends and the basidium begins.

This characteristic is similar to that of the genus *Chrysocyclis* Sydow (*Holwayella* Jackson). In *Chrysocyclis*, however, the spores are two-celled and the fully developed spore and basidium has a characteristic appearance which the writer has described as "mittens-like." *Chrysocyclis*, however, according to the writer's present interpretation, represents a lepto-*Puccinia* in which the sori are waxy and the basidium develops from the spore with no interruption. It is, therefore, questionable whether this character is sufficiently important to justify generic separation. To the writer it represents a natural simplification in morphology which has arisen in certain tropical rusts which germinate at once. The relatively thick firm walled teliospores with a definite pore, characteristic of nearly all rusts of temperate regions, may be interpreted as a morphological adaptation necessary to a resting condition. The absence of this characteristic does not necessarily justify generic separation.

#### SPECIES ON ROSACEAE

90. PHRAGMIDIUM DISCIFLORUM (Tode) J. F. James, Contr.  
U. S. Nat. Herb. 3: 276. 1895.

*Ascophora disciflora* Tode, Fungi Meckl. 1: 16, 1790.

*Rosa* sp. (cult.). San Felipe, Chile, Sept. 25, 1919. 73;  
Panamaruda, Chile, Dec. 16, 1919. 238; La Paz, Bolivia,  
March 20, 1920. 442.

91. PUCCINIASTRUM AGRIMONIAE (Schw.) Tranz. Scripta Bot.  
Hort. Univ. Petrop. 4: 301. 1895.

*Caeoma (Uredo) Agrimoniae* Schw. Trans. Am. Phil. Soc. II.  
4: 291, 1832.

*Agrimonia hirsuta* Bong. Campos do Jordão, São Paulo,  
Brazil, Apr. 25, 1922. 1770.

92. TRANZSCHELIA PUNCTATA (Pers.) Arth. Résult. Sci. Congr.  
Bot. Vienne 340. 1906.

*Aecidium punctatum* Pers. Ann. Bot. Usteri 20: 135. 1796.

*Puccinia Pruni-spinosae* Pers. Fung. 226. 1801.

*Prunus Persica* (L.) Stokes. Cochabamba, Bolivia, Feb. 26, 1920. 334; Hacienda del Urco, Urubamba Valley, Cuzco, Peru, July 4, 1920. 762.

## ON THE GENUS RUBUS

In connection with the study of the Holway rust collections on the genus *Rubus*, the writer has encountered several interesting situations and it seems desirable at this time to present a preliminary synopsis covering all the rust species occurring on the genus *Rubus* in Central and South America. In connection with this study the writer has also had available several collections made by Dr. F. L. Stevens in Ecuador and Peru. These together with other South American collections from various sources are also recorded, for the sake of completeness.

Among other things it has been discovered that the teliospores of *Spirechina Loeseneriana* and *S. Arthuri* occur in chains. This necessitates a transfer of these species to *Kuehneola*. Since *S. Loeseneriana* was designated the type of the genus *Spirechina* that generic name now becomes synonymous with *Kuehneola* and a new name is provided for the species with one-celled teliospores. We do this with the conviction that the relationship of the group of *Rubus* rusts characterized by one-celled teliospores is with *Kuehneola* and the Phragmidiatae and not with the Dicaeomatae. To leave them in *Uromyces* does not properly bring out this relationship.

In addition to the forms discussed here, *Kuehneola andicola* (Diet. & Neger) Diet. occurs in South America. We have not been able to assign properly *Uredo imperialis* Speg. because of the lack of authentic material.

93. ***Kuehneola Loeseneriana* (P. Henn.) Jackson & Holway, comb. nov.**

*Uredo Loeseneriana* P. Henn. Hedwigia 37: 373. 1898.

*Uromyces Usterii* Speg. Rev. Mus. La Plata 15: 7. 1908.

*Uromyces Loesenerianus* Sydow, Monog. Ured. 2: 202. 1910.

*Spirechina Loeseneriana* Arth. Jour. Myc. 13: 30. 1907.

*Rubus brasiliense* Mart. Barbacena, Minas Geraes, Brazil,  
Dec. 12, 1921, II. 1384.

*Rubus erythroclados* Mart. Campos do Jordão, São Paulo,  
Brazil, Apr. 22, 1922, II, III. 1752.

*Rubus floribundus* H.B.K. Hacienda La Florida, Prov. Sur  
Yungas, Bolivia, May 28, 1920. 673; San Felipe, Prov. Sur  
Yungas, Bolivia, May 19, 1920. 621.

*Rubus urticaefolius* Poir. São Paulo, Brazil, Jan. 25, 1922.  
1500; Santa Anna, São Paulo, Brazil, Feb. 21, 1922, II.  
1577.

*Rubus* sp. Therezopolis, Rio de Janeiro, Brazil, Sept. 29,  
1921, I, II, III. 1167; Petropolis, Rio de Janeiro, Brazil,  
Oct. 30, 1921. 1262; Bello Horizonte, Minas Geraes,  
Brazil, Nov. 26, 1921, II. 1341; Friburgo, Rio de Janeiro,  
Brazil, Jan. 2, 1922, I. 1443; Poços de Caldas, São Paulo,  
Brazil, Apr. 8, 1922, II, III. 1712; Campos do Jordão,  
São Paulo, Brazil, Apr. 20, 1922, I, III. 1739; Reserva  
Florestal, Itatiaya, Brazil, May 13, 1922, I, II, III. 1845.

93a. **Kuehneola Arthuri** (Sydow) Jackson, comb. nov.

*Uromyces Arthuri* Sydow, Monog. Ured. 2: 203. 1910.

*Spirechina Arthuri* Arthur, N. Am. Fl. 7: 183. 1912.

This species, as shown by the character of the markings on the urediniospores, is closely related to *K. Loeseneriana*. A careful study of the teliospores shows that they may occur in short chains though this is not easily demonstrated, as the spores soon separate.

*K. Arthuri* has apparently so far been reported only from Guatemala.

**MAINSIA** Jackson, gen. nov.

The generic name *Mainsia* is proposed as a substitute for *Spirechina* Arth. (Jour. Myc. 13: 30. 1907). This genus is needed to include those forms which show relationship to *Kuehneola*, the teliospores of which occur singly on short pedicels, not in chains. The name proposed is in honour of my former associate, Dr. E. B. Mains, whose contributions to uredinology are of far reaching importance.

The generic diagnosis is in general as given by Arthur for *Spirechina* (N. Am. Fl. 7: 182. 1912) with some modification. In these forms carefully studied by the writer the pycnia are not strictly subcuticular. Neither are they subepidermal but rather intra-epidermal. When the sori, whether pycnia, uredinia, or telia, occur on the upper surface, and possibly also when hypophyllous, the epidermal cells in the vicinity of the sorus are considerably hypertrophied so that the epidermis is considerably thicker in the infected region than elsewhere. The pycnia, uredinia, and telia develop as cavities in this hypertrophied epidermis and when nearly mature are covered by the cuticle and the upper epidermal wall. The side walls of the epidermal cells seem to be digested during the formation of the sorus.

The genus may include species which are microcyclic, as for example *M. Rubi-urtici* and *M. quitensis*. Paraphyses are present in some species. Most of the species appear to be of the brachy-type of life history. The primary uredinia usually occur on the upper surface, while the secondary uredinia and telia are usually on the under surface of the leaves.

To this genus should also be assigned the Asiatic species **Mainsia chinensis** (Diet.) Jackson, comb. nov. (*Spirechina chinensis* Diet. 1928), and the European **Mainsia urediniformis** (J. Mull.) Jackson, comb. nov. (*Uromyces urediniformis* (J. Mull.) Diet. 1912).

#### KEY TO THE AMERICAN SPECIES OF MAINSIA

Uredinia present, brachy-forms.

Urediniospore wall markings echinulate or verucose.

Markings of urediniospore wall coarse and sparsely placed.

Base of urediniospore markings elongated longitudinally..... 93b. *M. peruviana*.

Base of urediniospore markings rounded or nearly so. 94. *M. Holwayii*.

Markings of urediniospore wall rather fine.

Markings noticeably more prominent at apex.

Markings forming a distinct crown at apex..... 94a. *M. Pittieriana*.

- Markings not forming a crown  
at apex.
- Teliospores 24–32  $\mu$ . 94b. *M. Rubi.*
  - Teliospores 28–48  $\mu$  long. 97. *M. tenella.*
- Markings not noticeably more con-  
spicuous at apex.
- Teliospores thickened at apex;  
paraphyses present.
  - Urediniospores small, 20–  
26  $\mu$  long ..... 95. *M. Lagerheimii.*
  - Urediniospores large, 25–  
35  $\mu$  long ..... 96. *M. variabilis.*
  - Teliospores not thickened at  
apex; paraphyses absent.
  - Teliospores less than 50  $\mu$  long.
  - Urediniospores 24–30  $\mu$ ;  
telia hypophyllous. 97. *M. tenella.*
  - Urediniospores 20–24  $\mu$   
long; telia epiphyllous. .... 97a. *M. epiphylla.*
  - Teliospores more than 50  $\mu$   
long.
  - Teliospores broad, av-  
eraging more than  
18  $\mu$  ..... 97b. *M. Mayorii.*
  - Teliospores narrow, av-  
eraging less than  
18  $\mu$  ..... 98. *M. clara.*
- Urediniospore markings tuberculate ..... 98a. *M. cundinamarcensis.*
- Uredinia absent, microcyclic.
- Teliospores less than 50  $\mu$  long ..... 98b. *M. Rubi-urticifolii.*
  - Teliospores more than 50  $\mu$  long ..... 99. *M. quitensis.*

**93b. *Mainsia peruviana* Jackson, sp. nov.**

O. Pycnia epiphyllous, few, gregarious on yellowish spots,  
intra-epidermal, forming lenticular cavities in the hypertrophied  
epidermis.

II. Uredinia epiphyllous, grouped around the pycnia on yel-  
lowish spots, often arranged in a concentric manner, tardily  
naked, becoming pulverulent, long covered by the overarching  
outer epidermal wall; urediniospores obovate, 18–24 by 30–40  $\mu$ ;  
wall colorless, 2–3  $\mu$  thick, thickened at apex, 7–10  $\mu$ , the promi-  
nent sparsely verrucose markings elongated longitudinally; pores  
obscure.

III. Telia hypophyllous on the yellowish spots bearing the  
uredinia or scattered, round, whitish, pulvinate; ruptured epi-  
dermis not noticeable; teliospores clavate or cylindrical, 16–18

by 44–68  $\mu$ , germinating at once; wall uniformly thin, 1  $\mu$  or less, colorless; pedicel short, deciduous.

*Rubus* sp. Valle de Occobainbe, jede Utuma, Peru, Aug. 1922. Coll. Buez 861, comm. F. L. Herrera.

This very distinct species was sent me by Dr. B. O. Dodge. It is immediately separable from all other *Rubus* rusts of this group by the peculiarly distinct character of the markings on the urediniospore wall. These are in the nature of well separated rounded ridges, very sparsely placed. The ridges are parallel with the long axis of the spore and have a tendency to be arranged in lines. The species was not collected by the Holways.

#### 94. *Mainsia Holwayii* Jackson, sp. nov. (PLATE 11, A–C.)

O. Pycnia epiphyllous, few, gregarious on yellowish spots, intra-epidermal, forming lenticular cavities in the hypertrophied epidermis, 40–45 by 80–100  $\mu$ .

II. Uredinia epiphyllous, intra-epidermal, scattered or often arranged in a concentric manner around the pycnia, tardily naked, becoming pulverulent, long covered by the overarching outer epidermal wall; urediniospores obovate, 12–22 by 28–35  $\mu$ : wall colorless, 1.5–2  $\mu$ , thickened to 3·6  $\mu$  at apex, sparsely and rather prominently echinulate, the markings slightly more prominent at the apex; pores obscure.

III. Telia hypophyllous, scattered or gregarious, yellowish, pulvinate, compact, becoming whitish on germination; ruptured epidermis not noticeable; teliospores clavate, 12–16 by 48–70  $\mu$ , wall thin, 1  $\mu$  or less, colorless, not thickened at apex, germinating at once; pedicel short, colorless.

*Rubus floribundus* H.B.K. Hacienda "La Florida," Sur Yungas, Bolivia, May 26, 1920. 654 (type). May 28, 1920. 673a.

*Rubus urticaefolius* Poir. Chalhuapuguio, Peru, Dec. 8, 1924. F. L. Stevens 216, 222.

*Rubus* sp. Hacienda "Anacuri," Nor. Yungas, Bolivia, June 5, 1920. 720, 722.

A very distinct species characterized by the coarse, sparse, echinulate markings of the urediniospore wall which have little or no tendency to be elongated as in the preceding species. We

have placed tentatively with this species two collections made by F. L. Stevens on *Rubus urticaefolius* in Peru.

94a. **Mainsia Pittieriana** (P. Henn.) Jackson, comb. nov.

*Uromyces Pittierianus* P. Henn. Hedwigia Beibl. 41: 101. 1902.

*Uredo ochraceo-flava* P. Henn. Hedwigia Beibl. 41: 101. 1902.

*Spirechina Pittieriana* Arth. N. Am. Fl. 7: 183. 1912.

The characteristic arrangement of the markings of the urediniospore to form a crown at the apex distinguishes this species from all others. It is at present known only from Costa Rica. Specimens from South America in the Arthur herbarium which had been assigned to this species and the next are now referred to elsewhere in this paper.

94b. **Mainsia Rubi** (Dietel & Holway) Jackson, comb. nov.

*Uromyces Rubi* Dietel & Holway; Holway, Bot. Gaz. 31: 327. 1901.

*Spirechina Rubi* Arth. N. Am. Fl. 7: 184. 1912.

This species is now known on several species of *Rubus* from Central America. It has not been reported from South America.

95. **Mainsia Lagerheimii** (P. Magn.) Jackson & Holway, comb. nov.

*Uromyces andinus* Lagerh. Bull. Soc. Myc. Fr. 11: 213. 1895.

Not *U. andinus* P. Magn. 1893.

*Uromyces Lagerheimii* P. Magn. Ber. Deutsch. Bot. Ges. 14: 377. 1896.

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*Rubus bogotensis* H.B.K. Quito, Ecuador, Aug. 13, 1920. 884.

*Rubus floribundus* H.B.K. Valle de Chillo, Ecuador, Nov. 13, 1924. F. L. Stevens 289.

*Rubus* sp. Cuenca, Ecuador, Sept. 15, 1920. 987; Guapulo, Ecuador, Nov. 12, 1924. F. L. Stevens 257.

This species was originally described by Lagerheim from material collected at Quito, Ecuador, on *Rubus* sp. Mayor assigned two collections made in the mountains of Colombia on *Rubus glaucus* (Nos. 101, 302) to this species. We have not seen

Mayor's collections but the description fits our material admirably except that Mayor makes no mention of paraphyses. In Holway's No. 987 and in Stevens' collection 257 paraphyses are present in association with the telia. These are inconspicuous and irregular with thin walls evidently peripheral and incurved, 8–12 by 18–25  $\mu$ . This species is distinguished from the next by the smaller urediniospores and the more regular and less conspicuous thickening of the wall at the apex of the teliospore.

96. **Mainsia variabilis** (Mayor) Jackson & Holway, comb. nov.

*Uromyces variabilis* Mayor, Mem. Soc. Neuch. Sci. Nat. 5: 457. 1913.

*Spirechina variabilis* Diet. Die Nat. Pflanzenfamilien II auflage 6: 60. 1928.

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*Rubus megalococcus* Focke. Quito, Ecuador, Aug. 19, 1920.  
927.

*Rubus nubigenus* H.B.K. Quito, Ecuador, Aug. 19, 1920.  
925.

This characteristic species was based on a collection made by Mayor near Bogota, in Cundinamarca, Colombia (No. 301). The specimens listed above agree well with the description given by Mayor. *M. variabilis* is close to the preceding species but is separable on account of the large urediniospores and the more evident and irregular character of the thickened wall at the apex of the teliospore. Paraphyses are present in the telia similar to those mentioned in the preceding species.

97. **Mainsia tenella** Jackson & Holway, sp. nov.

O. *Pycnia epiphyllous*, gregarious on yellowish spots.

II. *Uredinia amphigenous*, chiefly epiphyllous, on yellowish spots, small, round, 0.4–0.6 mm. across, tardily naked, becoming pulverulent, long covered by the overarching epidermis; urediniospores ellipsoid or obovoid, 15–19 by 24–30  $\mu$ , rather variable in length; wall colorless, thin, 1.5–2.5  $\mu$ , sometimes slightly thickened at apex, finely and rather sparsely echinulate, the markings slightly more prominent on the upper half of the spore wall; pores obscure.

III. Telia few, hypophyllous, round, compact, whitish; ruptured epidermis not noticeable; teliospores variable, ellipsoid or

obovoid, 14–19 by 28–48  $\mu$ , rounded below and usually gradually narrowed above, germinating at once; wall colorless, uniformly thin, 1  $\mu$  or less; apical pore not evident; pedicel short, colorless.

*Rubus bogolensis* H.B.K. Huigra, Prov. Chimborazo, Ecuador, Aug. 2, 1920. 810 (type).

This species is nearest to *Mainsia Rubi* and perhaps to *M. clara*. It is distinguishable from both by the intermediate size of the teliospores. As in the latter, all the uredinia in our specimens occur in association with the yellowish spots bearing pycnia. The few telia that are present are also on these yellowish spots. The teliospores germinate at once and the apical pore is so delicate and undifferentiated that it is often difficult to determine when a spore has started to germinate.

The markings on the urediniospores are only slightly more prominent in the upper half of the spore and on this account the species is given in two places in the accompanying key.

97a. ***Mainsia epiphylla*** (Arth.) Jackson, comb. nov.

*Spirechina epiphylla* Arth. N. Am. Fl. 7: 184. 1912.

This species is known only from Texas on *Rubus trivialis*. The teliospores are described as being found in the epidermal cells. According to the interpretation of the writer this is not quite correct. As noted in the discussion of this genus the sori, including pycnia, uredinia, and telia, are often formed in intra-epidermal cavities. The epidermal cells in the vicinity of the sorus are often quite considerably hypertrophied and the sori form as cavities in this hypertrophied region. A careful study of the development of the sori in this genus made from fixed material would be highly desirable.

97b. ***Mainsia Mayorii*** Jackson, nom. nov.

*Uromyces quitensis* Mayor, Mem. Soc. Neuch. Sci. Nat. 5: 456. 1913. Not Lagerheim, 1895.

Mayor based his *Uromyces quitensis* on a specimen collected in Colombia on *Rubus* sp. (No. 301a). Through the courtesy of Dr. R. Thaxter we have been privileged to examine a portion of the type and find Mayor's description reasonably accurate.

We would give the urediniospore measurements 18–20 by 26–30  $\mu$ . The wall is 1.5–2  $\mu$  thick at the sides, occasionally thickened slightly at the apex to 3–3.5  $\mu$ . The markings are verrucose-echinulate and moderately spaced. The teliospores appear all to be germinated as described by Mayor and measure 18–22 by 50–75  $\mu$ . The wall is very slightly thickened at the sides near the pore. As explained elsewhere in this paper (No. 99) we would interpret *M. quitensis* Lagerh. as a lepto-form, and have therefore provided a new name for the species described by Mayor. Mayor included with his species three other collections bearing uredinia only, which we have not seen. This species is closest to *Mainsia clara* (No. 98) from which it is separable by its broader teliospores, which are slightly thickened at the apex, and the narrower urediniospores, also slightly thickened.

98. ***Mainsia clara* Jackson & Holway, sp. nov.**

- O. Pycnia epiphyllous, few, gregarious on yellowish spots.  
II. Uredinia chiefly hypophyllous, gregarious, small, round, tardily naked, pulverulent; ruptured epidermis conspicuous; urediniospores ellipsoid or obovoid, 20–24 by 30–36  $\mu$ ; wall 2–3  $\mu$  thick, colorless, moderately, uniformly, and rather finely, echinulate; pores obscure.  
III. Telia hypophyllous, round, 0.5–0.8 mm. across, usually associated with uredinia on yellowish spots, at first somewhat waxy, compact, whitish; ruptured epidermis not noticeable; teliospores obclavate or cylindric, 12–18 by 50–84  $\mu$ , rounded below, somewhat narrowed above; wall colorless, 1  $\mu$  or less thick, not thickened at apex, smooth; pedicel short, colorless.

*Rubus roseus* Poir. Sorata, Bolivia, Apr. 18, 1920. 543.

*Rubus* sp. Biblian, Prov. de Cañar, Ecuador, Sept. 8, 1920.

O, II, III, Holway 963 (type); Cuenca, Ecuador, Sept. 17–24, 1918. O, II, III, J. N. Rose 22832.

In our specimens of this species, any distinction between primary and secondary uredinia is quite impossible. Most, if not all, of the uredinia seen are on the under side of the yellowish spots which bear the pycnia, but whether they are all primary we have been unable to decide.

The species is readily distinguished from all others by the large, uniformly echinulated urediniospores taken together with the long teliospores and the absence of paraphyses.

We have placed with this species a collection made by Dr. J. N. Rose from the same region, which is in the Arthur herbarium labelled *Spirechina Rubi* (Diet. & Holw.) Arth. It is certainly not that species but agrees well with the one here described, except that the teliospores average somewhat narrower and longer. Another collection made by Rose on *Rubus boliviensis* Focke, at Loja, Ecuador, No. 23270, is tentatively placed here also. This collection consists of uredinia only and the spores while of the same general type average somewhat smaller than the ones here recorded. The urediniospores in all collections sometimes show a tendency to have the urediniospore markings slightly more prominent at the apex than at the base.

98a. **Mainsia cundinamarcensis** (Mayor) Jackson, comb. nov.

*Uromyces cundinamarcensis* Mayor, Mem. Soc. Neuch. Sci. Nat. 5: 452. 1913.

*Spirechina cundinamarcensis* Diet. in E. & P. Nat. Pfl. ed. II. 6: 60. 1928.

A very distinct species based on a single collection made in Colombia on *Rubus peruvianus* Fritsch (Mayor No. 105). It is reasonably well described by Mayor. The urediniospore wall is distinctly thickened at the apex and the markings are much more prominent at the apex than at the base. The most prominent distinguishing characters are the long, narrow teliospores and the tuberculate character of the urediniospore markings, especially those at the upper end of the spore. In recording the urediniospore size Mayor evidently did not give sufficient attention to the proper orientation of the spore. Our measurements gave 20-24 by 28-38  $\mu$  for the urediniospores and 11-19 by 85-110  $\mu$  for the teliospores.

We are tentatively assigning to this species a collection on *Rubus* sp. made near Las Juntas, Ecuador, by Dr. J. N. Rose, Sept. 28, 1918. No. 23198. This collection was found in the Arthur Herbarium labeled *Spirechina Pittieriana*. The rust in this collection is certainly closely related to the above, as shown by the character of the urediniospore. Only a few teliospores are present and these are old and germinated. They appear, however, to be considerably shorter and broader than in *U. cundi-*

*namarcensis*. We hesitate to describe it as new because of the meagre and unsatisfactory character of the material at our disposal.

98b. **Mainsia Rubi-urticifolii** (Mayor) Jackson, comb. nov.

*Uromyces Rubi-urticifolii* Mayor, Mem. Soc. Neuch. Sci. Nat. 5: 454. 1913.

From Mayor's description one would judge that this species is a lepto-form. It is based on several collections made in the mountains of Colombia on *Rubus urticifolius* by Mayor. We have seen but one of Mayor's specimens. The teliospores are considerably shorter than in the next species (*M. quitensis*).

99. **Mainsia quitensis** (Lagerh.) Jackson & Holway, comb. nov.

*Uromyces quitensis* Lagerh. Bull. Soc. Myc. France, 11: 213. 1895.

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*Rubus bogotensis* H.B.K. Quito, Ecuador, Aug. 13, 1920, III. 883.

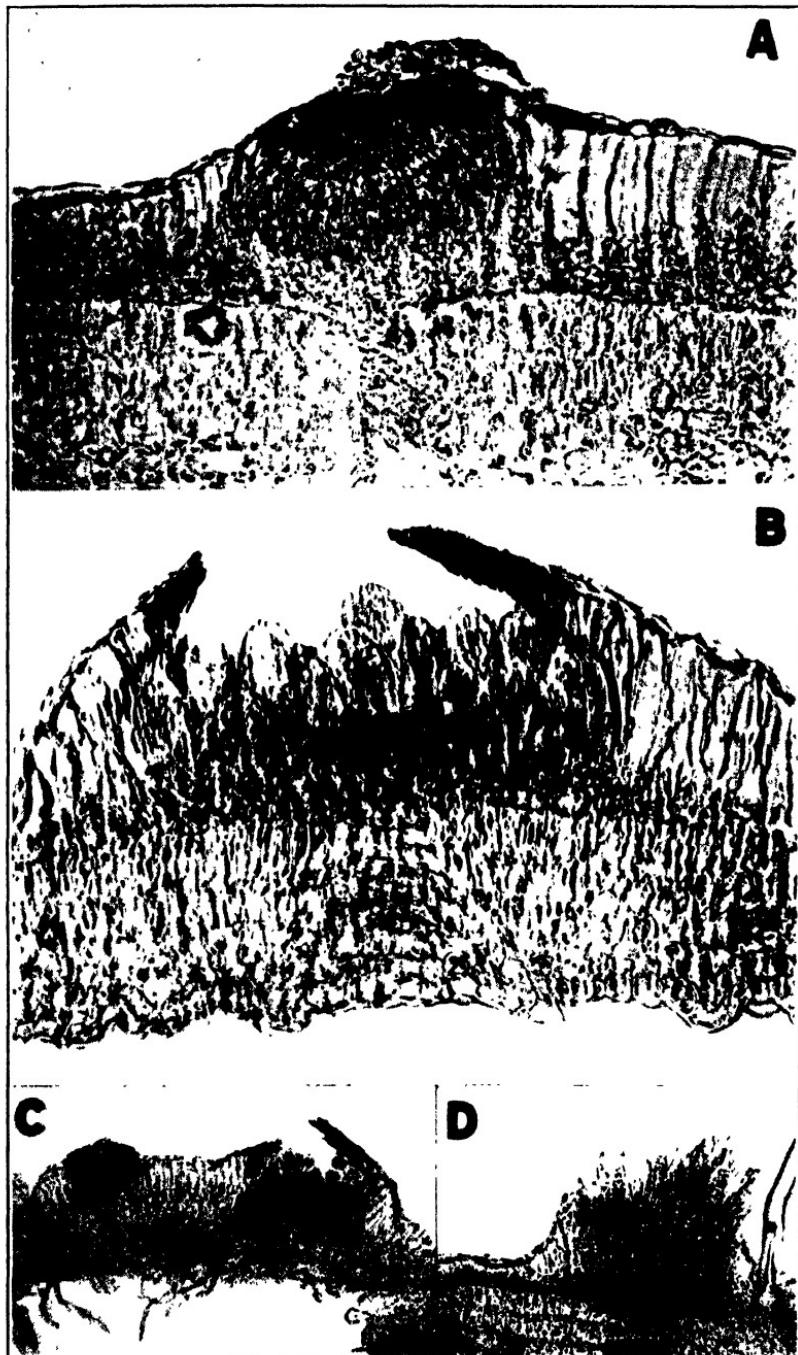
*Rubus floribundus* H.B.K. San Felipe, Sur Yungas, Bolivia, May 19, 1920, III. 617.

This species was described by Lagerheim from material collected on *Rubus* sp. near Quito, Ecuador, but has not been otherwise reported except by Mayor from Colombia. It was described as a lepto-form and, while we have not seen the type specimen, the two collections listed above conform with the description given by Lagerheim, and one of them is from the type locality. Our specimens are quite certainly short cycled. The telia occur on both the upper and lower surfaces of the leaves on yellowish spots. There are no pycnia.

Mayor assigned four collections which he obtained in Colombia to this species. Mayor's description, however, includes uredinia as well as telia. According to our interpretation Mayor was in error in assigning his collections to *U. quitensis* and we have, therefore, provided a new name for the species as described by him (*M. Mayorii*. No. 97b).

## EXPLANATION OF PLATE 11

*a, b, c. Mainsia Holwayii.* Pycnia and primary uredinia showing relation to hypertrophied upper epidermal cells. *d. Mainsia quitensis.* Showing relation of telia to hypertrophied upper epidermis. Photomicrographs from free hand sections.



MAINSIA HOLWAYII



# DIAGNOSES OF AMERICAN PORIAS—III.<sup>1</sup> SOME ADDITIONAL BROWN SPECIES, WITH A KEY TO THE COMMON BROWN SPECIES OF THE UNITED STATES AND CANADA

L. O. OVERHOLTS

(WITH PLATES 12-14)

This paper presents descriptions and illustrations of five additional *Poria* species, not previously involved in my reports on this extremely difficult genus. This presentation is followed by a key to some common species of brown *Poria* and resupinate conditions of species of *Trametes*, *Fomes*, and *Polyporus* that are of common occurrence and that are likely to be looked for in the genus *Poria*. Unfortunately there is no known method of distinguishing such resupinate forms of normally pileate species from true species of the genus *Poria*.

**PORIA FERREA** Pers. Myc. Eur. 89. 1825. (PLATE 12, FIG. 5; PLATE 13, FIGS. 10, 12.)

*Mucronoporus fulvidus* Ellis & Ev. Proc. Phil. Acad. Nat. Sci. 1894: 323. 1894.

Sporophore perennial only for a maximum of four to five years, effused, sometimes in elliptical patches 5-7 × 2-3 cm., sometimes for several centimeters, in age seeming to loosen from the substratum to some extent, typically with a sterile, cinnamon-colored, fibrillose-tomentose margin that may be quite regular but often irregular and somewhat nodulose; subiculum quite thin though fairly conspicuous in young plants and about 0.5 mm. thick, eventually indistinct; tube layers reaching a total thickness of 0.6-0.8 cm., the tubes 1-3 mm. long each season, whitish within; pore surface cinnamon-brown to dark-brown or sometimes rusty brown, weathering to grayish in age, the mouths subcircular to somewhat angular, thick-walled, entire, averaging 4 to 5 per mm.; spores cylindric, hyaline, smooth, 5-7 × 2-2.5  $\mu$ ;

<sup>1</sup> Contribution from the Department of Botany, The Pennsylvania State College, No. 74. Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station, as Technical Paper No. 501.

basidia 5–6  $\mu$  diameter; setae rather abundant, projecting 10–30  $\mu$  beyond the basidia, gradually attenuate to a narrow sharp tip, 5–10  $\mu$  diameter at the base; imbedded setal hyphae absent; subiculum hyphae flexuous, reddish-brown in KOH, mostly simple, with no cross walls or clamps, 2–3  $\mu$  diameter, a few paler hyphae sometimes with branches and cross walls.

On dead wood of deciduous trees, especially on *Alnus*, but also on *Acer*, *Castanea*, *Quercus*, and perhaps other species.

Specimens examined: Greenwood Furnace and Garner's Run, Huntingdon Co., Pa. (nos. 10747, 11010); Emerson, Vermilion, and Stevens, Mich. (nos. 9102, 5828, 11769); Corvallis, Ore. (11935); Berkeley, Calif. (type of *M. fulvidus* in The New York Botanical Garden Herbarium).

Though originally described as with globose spores 2  $\mu$  diameter, an examination of the type of *M. fulvidus*, of European specimens from Romell, and of collections in my own herbarium shows that they are cylindric as described by Romell.

On small limbs this species has the habit of *P. inermis*, forming elongate or elliptic sporophores that become quite thick for their width, and with age the marginal portion dies, recedes, becomes tumid, and tends to loosen from the substratum. The types of *M. fulvidus* were collected on *Alnus* and that may prove to be its more usual host.

The coloration is rather unusual among species of brown *Poria* which are typically yellow-brown to darker brown. A dark-cinnamon or gilvoid color predominates here.

My interpretation of this species is based on specimens communicated to me by Romell, who reports that they agree with specimens determined by Persoon.

**PORIA PRUNICOLA** (Murr.) Sacc. & Trott in Sacc. Syll. Fung. 21: 331. 1912. (PLATE 12, FIGS. 2, 6; PLATE 13, FIG. 8.)

*Fomitiporia prunicola* Murrill, N. Am. Fl. 9: 9. 1907.

Sporophore perennial, first appearing in circular patches 1–3 cm. diameter, finally rather widely effused, thin specimens with a tendency to loosen slightly on the margin, otherwise adnate, at first with a narrow yellow-brown or rusty-brown tomentose border that disappears in older specimens; subiculum quite thin, inconspicuous, brown, finally largely disappearing; tube layers

reaching a total thickness of about 1 cm. but usually thinner, the tubes 1-2 mm. long each season, not whitened within except in the old layers, rather indistinctly stratified; pore surface rusty brown to "buffy brown" (Ridgway), becoming somewhat grayish after long weathering, the mouths subcircular, thick-walled, entire, averaging 4 to 6 per mm.; spores broadly ellipsoid or subglobose, smooth, hyaline,  $4-5 \times 3-4 \mu$ ; basidia  $4-6 \mu$  diameter; setae present, rather rare, scarcely projecting beyond the basidia, sharp-pointed,  $15-20 \times 4-6 \mu$ ; hyphae brownish-red in KOH, simple, no cross walls or clamps,  $3-4 \mu$  diameter.

On dead wood of *Prunus*.

Specimens examined: Medford, Me. (Type in The New York Botanical Garden Herbarium); Crawford Notch, N. H. (no. 4580); Phoenix, Lewis, Cranberry Lake, N. Y. (nos. 2579, 7066, 5857); Vermilion, Mich. (no. 5827); in Canada from Winnipeg, Manitoba (no. 7054).

Some would probably prefer to regard this as a small-spored form of *Fomes igniarius* var. *luevigata*, but young specimens look very different. In old specimens there is a similar whitening of the old tubes as in that species. The small narrow setae and the subglobose hyaline spores on basidia not more than  $4-6 \mu$  diameter will separate this species from most of the related brown ones.

PORIA PUNCTATA Fries, Hymen. Eur. 572. 1874. (PLATE 12,  
FIG. 3; PLATE 14, FIGS. 15, 17.)

*Poria Friesiana* Bres. Ann. Myc. 6: 40. 1908.

*Fomitiporia laminata* Murrill, N. Am. Fl. 9: 11. 1907.

*Poria laminata* (Murr.) Sacc. & Trott, Sylloge Fung. 21: 336. 1912.

*Fomitiporia obliquiformis* Murrill, N. Am. Fl. 9: 9. 1907.

Sporophore perennial, effused for several centimeters, never loosening from the substratum, sometimes cracking on drying, young specimens with a rather distinct yellow-brown tomentose margin that mostly disappears with age; subiculum a thin brown inconspicuous membrane that largely disappears in old plants; tube layers reaching a total thickness of 1.5 cm., the tubes 1-3 mm. long each season, soon stuffed with a rusty gray or gray mycelium, the annual layers separated by distinct though extremely narrow layers of subiculum so that the region is distinctly though inconspicuously stratified; pore surface yellow

brown to "buffy-brown" or "Saccardo's umber" (Ridgway), the mouths circular to sub-circular, thick walled, entire, averaging 6 to 8 per mm.; spores globose or subglobose, smooth, hyaline, 5-8  $\mu$  diameter; basidia much inflated, 7-10  $\mu$  diameter; setae none; cystidia (?) usually abundant as finely attenuate hyaline hyphae less than 2  $\mu$  diameter, enlarged to bulbous or inflated bases, projecting 10-30  $\mu$ ; hyphae long and flexuous, brown, no cross walls or clamps, diameter 2-4  $\mu$ .

On dead wood of deciduous trees; noted on *Acer*, *Alnus*, *Asimina*, *Betula*, *Carpinus*, *Cephalanthus*, *Fraxinus*, *Maclura*, *Ostrya*, *Oxydendrum*, *Pyrus*, *Robinia*, *Quercus*, and *Salix*, with particular preference for *Salix*.

Specimens examined: Piscataquis Co., Me. (Type of *P. laminata* in The New York Botanical Garden Herbarium); Lake Winnipesaukee, N. Conway, and Temple, N. H. (nos. 5957, 5169, 9051); Syracuse, Norwich, Silver Lake, and Ithaca, N. Y. (nos. 5825, 8053, 8051, 3973); Detwiler Run, Huntingdon Co., Pa. (no. 9732); Morgantown, W. Va. (no. 7132); Clark Co., Ga. (no. 9571); Berea, Ky. (no. 9270); Oberlin, Ohio (no. 8539); Weirton and Turkey Run State Park, Ind. (no. 3928, 11810); Hillsboro, Ill. (no. 9162); Dexter (2), New Richmond, Ann Arbor (2), and Whitmore Lake, Mich. (nos. 9090, 9155, 9156, 8658, 9159, 8660); Balsam Lake, Wis. (no. 9053); Rush City, Minn. (no. 7065); Meramec Highlands (2), Wicks (2), Creve Coeur Lake, Perryville (3), and Jefferson Barracks, Mo. (nos. 5721, 2571, 2865, 2586, 5710, 2964, 2691, 2737, 5729); in Canada, from Winnipeg, Manitoba (no. 6152), and Edmonton, Alberta (no. 12095).

This species seems to be common where extensive collections have been made from New Hampshire to Wisconsin and Missouri. The early American mycologists referred it to *Fomes salicinus* and *Poria obliqua*, for the most part. Bresadola states that in Europe it was known as var. *resupinatus* of *Fomes igniarius*, and as *Poria contigua*. While it may be *P. contigua* of Fries, it is very improbable that it is *P. contigua* Pers. from which, as well as from *P. ferruginosa*, *Trametes tenuis* and related species, it differs in the lack of setae. Bresadola on this point says that in European specimens (discussed as *P. Friesiana* Bres.) setae are very rare and in a specimen from Romell I find some setae

present. I have sectioned numerous American collections, however, without finding a trace of setae. Otherwise the species is characterized by the plainly laminated hymenial region, the globose spores, the large basidia, and the numerous cystidia-like organs usually present in the hymenium. When one compares first-year stages with those several years old, they appear very different, but a large series of specimens renders these differences of no taxonomic value. Murrill describes *P. obliquiformis* as not having tubes definitely stratified; yet his specimens scarcely justify this statement. Hence I am referring to this species all collections with definitely stratified tubes soon whitish within, with globose spores 5–8  $\mu$  diameter, large basidia 7–10  $\mu$  diameter, and an entire lack of setae (in American specimens). The cystidia-like bodies usually present in the hymenium may not be of diagnostic value. In old specimens the margin gradually recedes and the outermost portions often become blackish or dark gray with age.

**PORIA TSUGINA** (Murr.) Sacc. & Trott in Sacc. Syll. Fung. 21: 332. 1912. (PLATE 12, FIG. 4; PLATE 14, FIGS. 14, 16.)

*Fomitiporia tsugina* Murrill, N. Am. Fl. 9: 9. 1907.

Sporophore perennial, rather widely effused, adnate to the substratum, the first year with a rather broad thin sterile rusty-yellow margin, this less conspicuous in succeeding years until it entirely disappears, the margin finally thicker and darker in color; subiculum as a thin brown membrane not more than 1 mm. thick; tube layers reaching a total thickness of 6 cm., but often 1 cm. or less, the tubes 4–8 mm. long each season, rather distinctly stratified, typically golden-brown where cut vertically but somewhat grayish within, especially in age; pore surface dark golden-brown or tawny to dark grayish-brown, the mouths subcircular to subangular, thick-walled, entire, typically averaging 6 to 8 per mm., sometimes somewhat larger; spores globose or subglobose, smooth, hyaline, 5–7  $\mu$  diameter; basidia inflated, 8–10  $\mu$  diameter; setae none; hyphae brown, simple, no cross walls or clamps, diameter 2.5–3.5  $\mu$  diameter.

On dead wood of coniferous trees; noted on *Picea*, *Pinus*, and *Tsuga*.

Specimens examined: Hebron, Lisbon, and N. Conway, N. H. (Type in The New York Botanical Garden Herbarium, and nos.

5091, 4962); Jamesville, Phoenix and Vaughns, N. Y. (nos. 3223, 2575, 4788); Laurel Run, Reitz Gap, Stone Valley (2), Shaver's Creek, and Warriors Mark, Pa. (nos. 11004, 5439, 5991, 5893, 7331, 6078); New Brunswick (2), N. J. (nos. 5674, 7850); Lorain Co., Ohio (no. 9783); Eldora, Colo. (no. 9167); Priest River, Ida. (no. 4516); Multnomah Co., Ore. (no. 7135); Requa and Korbel, Cal. (nos. 9052, 4719).

In Pennsylvania this is a rather frequent species, usually on *Tsuga* logs. It frequently has the habit of growing on the lower side of limbs 2 or 3 inches diameter on dead snags, and often where the dead branch joins the trunks. In such situations, after a few years of growth with the unusually long tubes it has somewhat of a pendant appearance as shown in Plate 14, figure 14. However it is frequently found on the lower side of large fallen logs.

In one collection I have noted a trace of a pale brown coloration in the walls of the spores, but otherwise they are hyaline.

Perhaps the species is too closely allied to *Fomes robustus* as indicated by the typical bright brown or golden brown color within, the long tubes fairly well stratified, the minute pores, the large basidia, large globose spores 6-8  $\mu$  diameter, and the lack of setae.

**PORIA WEIRII** Murrill, Mycologia 6: 94. 1914. (PLATE 12,  
FIGS. 1, 7; PLATE 13, FIGS. 9, 11, 13.)

***Fomitiporia Weirii*** Murrill, Mycologia 6: 93. 1914.

Sporophore perennial for a maximum of five or six years, light of weight and soft of texture when compared with most perennial species, effused for several centimeters, separating rather readily from the substratum, the first year's growth with a broad (1-2 mm.) fibrillose, sterile margin of which, due to irregularities of growth, little remains in older plants; subiculum brown, soft, punky, usually only 1-2 mm. thick but thicker at times due to irregularities in the substratum; tube layers reaching a total thickness of 2 cm. but often less than 1 cm., the tubes 2-4 mm. long each season, not at all whitened within, the annual layers separated by a thin soft subicular membrane and easily loosening from each other; pore surface dark umber brown to dark rusty-brown, the mouths angular, at maturity very thin walled but entire, averaging 5 to 6 per mm.; spores globose or subglobose,

smooth, hyaline, 4–6  $\mu$  diameter; setae usually very abundant, projecting very conspicuously, tapering to a sharp point, usually somewhat incrusted, 6–12  $\mu$  diameter, and readily seen to be the termini of large brown imbedded hyphae; imbedded setal hyphae rather numerous in the walls of the tubes, 6–12  $\mu$  diameter; hyphae of the subiculum thin walled, sparingly branched, with rather abundant cross walls, no clamps, the branches originating at a point median between two adjacent cross walls, 2.5–6  $\mu$  diameter.

On coniferous wood, perhaps largely or entirely confined to *Thuja plicata*.

Specimens examined: Priest River and Upper Priest River, Idaho (nos. 3927, 11842, 3525, 11886, 11883); Waltersville, Ore. (no. 8208).

Although a large series of specimens have been examined from the northwest, this species has appeared but once except in Idaho collections.

It is a well marked species with its light weight, spongy consistency, the separating annual tube layers, and above all in the peculiar setae that are found in very few other American *Poria*. The major portion of the tube walls is made up of large setal hyphae 6–12  $\mu$  diameter at their tips. Some of these tips are imbedded in the tissue of the walls but most of them curve into the hymenial layer and become the projecting setae of that region. The ordinary hyphae of the subiculum and the walls are also quite characteristic in their thin exterior walls, with rather numerous cross walls, no clamps, and the branches originating at a point midway between two adjacent walls.

In connection with the original description, Murrill recorded some notes from Weir to the effect that the species is at times parasitic on *Thuja plicata* and is at any rate an important timber decaying organism.

#### A KEY TO THE COMMON BROWN SPECIES OF *PORIA* IN THE UNITED STATES AND CANADA

The study of the various species of *Poria* may be much facilitated when all are correctly known, but their accurate identification will always be a problem to tax the keenest mycological student. At the present time we are not sure just which char-

acters are reliable and which ones subject to too much variation to be reliable for specific identification. We are certain that great reliance, here as in other fungi, can be placed on the spores produced on the basidia. In the brown species the presence or absence of setae and the form of these organs are of considerable importance. The size of the basidia affords an item of diagnostic value not hitherto recognized. One section of brown species has basidia 8-10  $\mu$  in diameter, and consequently they are conspicuous when the sporophore is in fruiting condition. Another section has small basidia 3-6  $\mu$  in diameter. Large globose spores 6-8  $\mu$  diameter are correlated with basidia of large diameter, and small globose or cylindric spores with basidia of small diameter. In most species the hyphae that make up the sporophore are not highly characteristic, but in a few cases the lack of thickened hyphal walls and the presence of cross walls in the hyphae are great aids in specific diagnoses. Of superficial characters that pertain to brown species, probably in general the less reliance that is placed on them the better. A distinction into annual and perennial forms can be made but is often misleading. The extent to which definite layering of tubes is present in perennial forms is of some importance; the thickness of the sporophore produced is of less value. Substratal habitats often give a clue to identification but may be misleading.

Thus it is evident that the leading characters on which species may be based are mainly microscopic. This is to be expected in a genus in which the sporophore is without pileus or stem. The same situation faces the student of any group of resupinate Basidiomycetes. Therefore, it is useless to attempt identification without recourse to free-hand sections of the tubes, preferably cut crosswise to the long axis of the tubes. The thinner these sections can be made, the better. Mounting in lactic acid is often preferable to mounting in KOH, which causes the sections to become very dark.

No species are regarded as belonging to the section of the true brown *Poria* unless they show a characteristic darkening or blackening when the sections are immersed in KOH or when KOH solution is applied directly to the tubes. Further, I have included in the following key the species of *Polyporus*, *Fomes*,

and *Trametes* that are likely to be found in a resupinate condition. The key is followed by a list of the brown species so far presented in my various papers on this genus, together with page references to the descriptions already presented. Many collections yet remained unnamed in my herbarium, and a number of other species have been described from this area. Hence the following key is offered only as a summary of facts relating to the species that now seem to stand out as distinct and at the same time as rather common members of our flora.

1. Setae absent when cross-sections of the tubes are examined ..... 2  
Setae present though not necessarily abundant in cross-sections of the tubes ..... 6
2. Growing only on wood of coniferous trees ..... 4  
Growing only on wood of deciduous trees ..... 5
3. Growing only on charred wood ..... *Trametes carbonaria*.  
Not growing on charred wood ..... 4
4. Sporophore thin, soft, and pliant, separating from the very rotten substratum as a thin pliant sheet; tubes rarely stratified in two or three layers; spores ellipsoid to subglobose,  $5-7 \times 3-5 \mu$ ; hyphae of subiculum thin-walled,  $5-7 \mu$  diameter, with frequent cross walls; mouths of the tubes averaging 1 to 2.5 per mm. .... *P. subiculosa*.  
Sporophore leathery to woody, not pliant, and not so separating; tubes not distinctly stratified; spores cylindric,  $9-12 \times 3-5 \mu$ ; hyphae of subiculum  $3-6 \mu$  diameter, without cross walls; mouths of the tubes averaging 2 to 3 per mm. .... *Trametes odorata*.  
Sporophore woody, soon 1 to several centimeters thick, not separating; tubes in distinct annual layers; spores globose,  $6-7 \mu$  diameter; hyphae of subiculum without cross walls,  $2.5-3.5 \mu$  diameter; mouths of the tubes averaging 6 to 8 per mm. .... *Poria tsugina*.
5. Spores brown,  $4-5 \mu$  diameter; tubes not stratified; pores averaging 3 to 4 per mm.; basidia  $5-6 \mu$  diameter; usually on *Ilex* or *Nemopanthes*.  
*P. inermis*.  
Spores hyaline,  $5-8 \mu$  diameter; tubes more or less distinctly stratified; pores averaging 6 to 8 per mm.; basidia  $7-10 \mu$  diameter; not on *Ilex* or *Nemopanthes* but on a great variety of other hardwoods. .... *P. punctata*.
6. Growing on wood of coniferous trees ..... 7  
Growing on wood of deciduous trees ..... 10
7. Plants showing one of the following sets of characters:
  - A. With large brown setal hyphae  $6-12 \mu$  diameter making up most of the tissue of the walls of the tubes<sup>2</sup> and ending in large protruding hymenial setae (see fig. 9 of this paper) ..... 8
  - B. As in the above but setal hyphae less conspicuous, only  $4-6 \mu$  diameter; hymenial setae not incrusted. .... *Polyporus glomeratus*.
  - C. Setae lanceolate, projecting  $25-60 \mu$  beyond the basidia; no imbedded setal hyphae; spores cylindric. .... *Trametes tenuis*.

<sup>2</sup> Best seen in thin longitudinal sections of the tubes.

- D. With a narrow black line in the subiculum (conjust) text below the tube layer; no setal hyphae; setae projecting less than  $25 \mu$  beyond the basidia; spores cylindric. *Fomes nigrolimitatus*.  
 Plants not fitting well into any of the above ..... 9
8. Sporophore truly perennial, the annual tube layers separated by a thin subicular membrane and easily loosening from each other; spores globose,  $4-6 \mu$  diameter ..... *Poria Weirii*.
- Sporophore sometimes perennial for two or three seasons, the tubes not layered and not separating; spores cylindric,  $3-5 \times 1-2 \mu$ .  
*P. ferrugineo-fusca*.
9. Setae  $6-7 \mu$  diameter at base; pores averaging 5 to 7 per mm.  
*Fomes putearius*.  
 Setae  $9-15 \mu$  diameter at base; pores averaging 3 to 5 per mm. *Fomes Pini*.
10. Sporophore annual or rarely reviving a second season ..... 11  
 Sporophore truly perennial with downward extension of the tubes year by year ..... 13
11. With imbedded setal hyphae (as under 7A) ..... *Polyporus glomeratus*.  
 Without such imbedded setal hyphae ..... 12
12. Pores averaging 6 to 8 per mm.; spores  $4-6 \times 2.5-3.5 \mu$ ; setae projecting less than  $25 \mu$  ..... *Polyporus gilvus*.  
 Pores averaging 4 to 6 per mm.; spores  $4-6 \times 2.5-3.5 \mu$ ; setae projecting less than  $30 \mu$  ..... *Poria ferruginosa*.  
 Pores averaging 1 to 3 per mm.; spores  $6-7 \times 2-2.5 \mu$ ; setae projecting  $25-60 \mu$  ..... *Trametes tenuis*.
13. Growing only on *Prunus* ..... 14  
 Growing on other substrata ..... 15
14. Hyphae with cross walls as seen in crushed mounts, diameter  $3-6 \mu$ ; setae  $6-8 \mu$  diameter; chiefly on *Prunus* species of the plum group.  
*Fomes fulvus*.  
 Hyphae without cross walls, diameter  $3-4 \mu$ ; setae  $4-6 \mu$  diameter; chiefly on *Prunus* species of the cherry group ..... *Poria prunicola*.
15. Pores averaging 4 to 5 per mm.; spores cylindric,  $5-7 \times 2-2.5 \mu$ .  
*Poria ferrea*.  
 Pores averaging 4 to 5 per mm.; spores globose, hyaline,  $5-6.5 \mu$  diameter.  
*Fomes igniarius* var. *laevigatus*.  
 Pores averaging 6 to 8 per mm.; spores hyaline,  $4-5 \times 3-4 \mu$ .  
*Fomes conchatus*.  
 Pores averaging 8 to 10 per mm.; spores pale rusty,  $3-3.5 \mu$  diameter.  
*Fomes densus*.

LIST OF COMMON SPECIES OF BROWN PORIA, WITH SOME SYNONYMY, AND WITH REFERENCES TO DESCRIPTIONS IN PREVIOUS PAPERS

BETULINA (PORIA). A synonym for *Fomes igniarius* var. *laevigatus* Fries.

CARBONARIA (TRAMETES). Originally described as *Hexagonia* (Grevillea 1: 68. 1872). Described by Murrill (N. Am.

Fl. 9: 4. 1907) as *Fuscoporia*. Known from N. Y., S. Car., Idaho, Mont., Oreg., and Calif. Usually on charred *Sequoia* logs in the west. Occasionally pileate, hence not a true *Poria*. *T. Sequoiae* Copeland is a synonym. Not previously described in this series.

**CONCHATUS** (FOMES). Rarely occurs entirely resupinate, usually with some indication of a pileus. Described in all the manuals. Ranges from Maine to Florida and west to Montana and Texas. Also in Canada. On a variety of hardwoods, never on conifers.

**DENSUS** (FOMES). A species described by Lloyd (Synopsis of Genus *Fomes* 245. 1915). Previously referred to *F. conchatus*. Usually resupinate. Known from Ohio, Indiana, Missouri, and Iowa. On hardwood logs. Not described in this series of papers.

**FERREA** (PORIA). A true *Poria*. *P. fulvida* Ellis & Ev. is a synonym. See p. 117 of this paper.

**FERRUGINEO-FUSCA** (PORIA). A true *Poria*. *P. marginella* (Peck) Sacc. is a synonym. See N. Y. State Mus. Bull. 205-206: 88. 1919.

**FERRUGINOSA** (PORIA). A true *Poria*. See Mycologia 14: 5. 1922. *P. Macounii* Peck is a synonym.

**FULVIDA** (PORIA). A synonym for *P. ferrea* Pers., which see.

**FULVUS** (FOMES). Rarely occurs entirely resupinate. Described in all the manuals. Ranges from Maine to Alabama and west to Montana. On species of *Prunus* belonging to the plum section.

**PINI** (FOMES). Occasionally occurs nearly or quite resupinate. Described in all the manuals. On coniferous wood only. Ranges throughout the United States and Canada.

**PRUNICOLA** (PORIA). A true *Poria*. See p. 118 of this paper.

**PUNCTATA** (PORIA). A true *Poria*. See p. 119 of this paper. *P. laminata* Murrill and *P. obliquiformis* Murrill are synonyms.

**PUTEARIUS** (FOMES). Usually occurs more or less resupinate. Originally described by Weir in Jour. Agr. Res. 2: 163. 1914, but spores are hyaline,  $4-5 \times 3-4 \mu$  (not "colored, 7-8  $\mu$ "). On coniferous woods only. Known only in Idaho, Montana, Oregon, and Washington.

**SEQUOIAE (TRAMETES).** A synonym for *T. carbonaria* Berk. & Curt., which see.

**SETIGERA (PORIA).** Probably a young stage of *Polyporus glomeratus* Peck. See Bull. N. Y. State Mus. 205-206: 109. 1919.

**SETOSUS (TRAMETES).** A synonym for *T. tenuis* Karst., which see.

**SUBICULOSA (PORIA).** A true *Poria*. See Bull. N. Y. State Mus. 205-206: 115. 1919.

**SUPERFICIALIS (PORIA).** A synonym for *T. tenuis* Karst., which see.

**TENUIS (TRAMETES).** Usually occurs entirely resupinate. *T. setosus* Weir (Jour. Agr. Res. 2: 164. 1914), *P. superficialis* (Schw.) Cooke, and *P. viticola* (Schw.) Cooke are synonyms. See also Mycologia 15: 224, 225. 1923, for descriptions.

**TSUGINA (PORIA).** Probably a true *Poria*. See p. 121 of this paper.

**VITICOLA (PORIA).** A synonym for *T. tenuis* Karst., which see.

**WEIRII (PORIA).** A true *Poria*. See p. 122 of this paper.

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#### EXPLANATION OF PLATES

##### PLATE 12

Fig. 1. *Poria Weirii*. Hyphae from the subiculum, to show prevalence of cross walls and manner of branching. No. 3927.  $\times 820$ .

Fig. 2. *Poria prunicola*. Camera lucida sketch to show prevalence of setae in the hymenium. From type specimen.  $\times 170$ .

Fig. 3. *Poria punctata*. Drawing of small portion of the hymenium to show basidia, spores, and the characteristic cystidia-like organs usually present. No. 8658.  $\times 820$ .

Fig. 4. *Poria tsugina*. Drawing of small portion of the hymenium to show basidia and spores. No. 5893.  $\times 820$ .

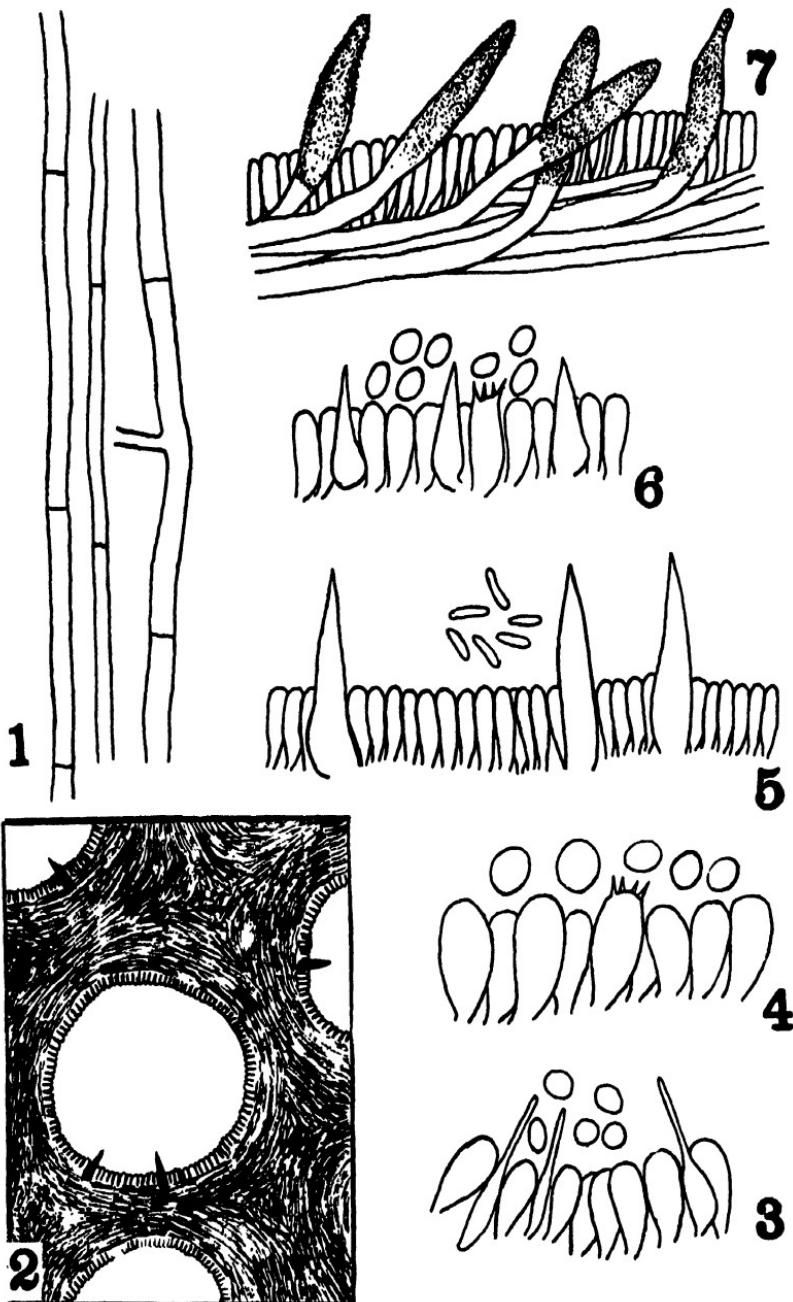
Fig. 5. *Poria ferrea*. Drawing of small portion of the hymenium to show basidia, setae, and spores. No. 10747.  $\times 820$ .

Fig. 6. *Poria prunicola*. Drawing of small portion of the hymenium to show basidia, setae, and spores. From type specimen.  $\times 820$ .

Fig. 7. *Poria Weirii*. Drawing of small portion of the hymenium to show the incrusted setae and their origin at the tips of large hyphae that extend into the trama of the tubes. No. 3927.  $\times 820$ .

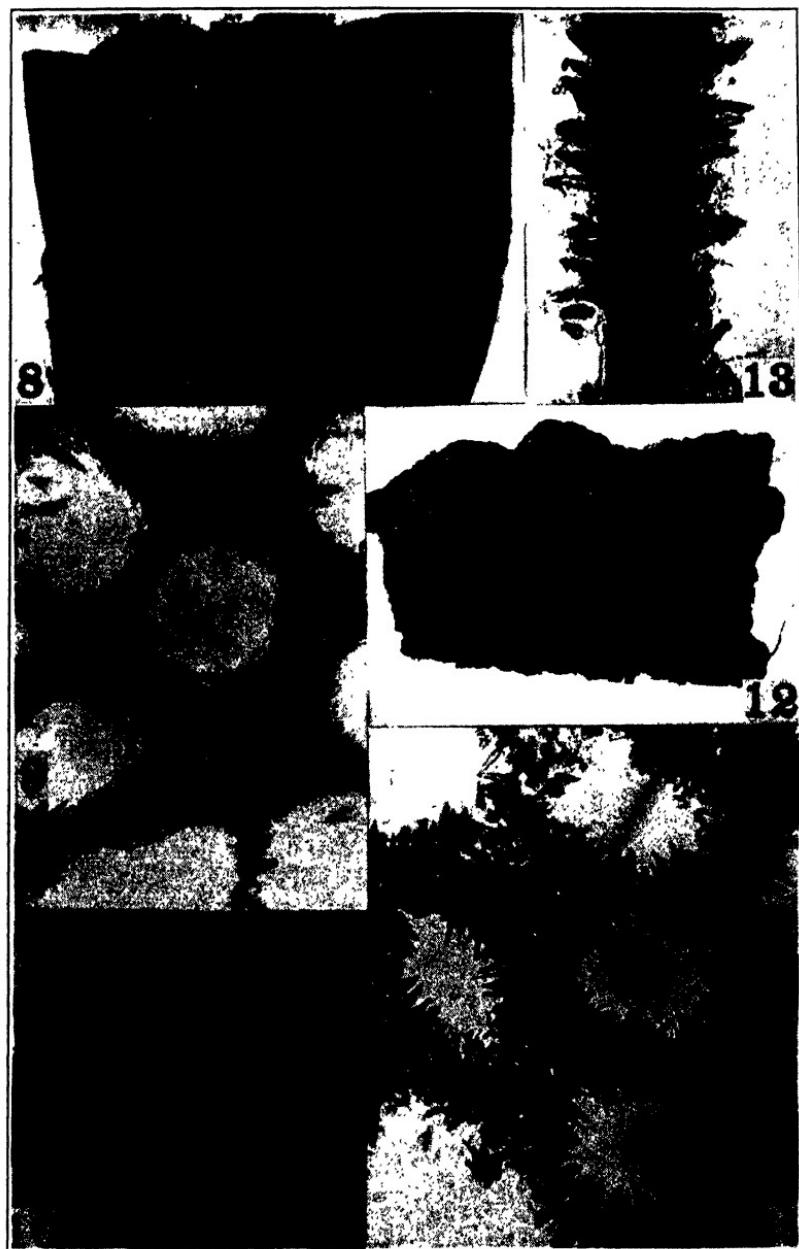
##### PLATE 13

Fig. 8. *Poria prunicola*. Photo of typical specimens in first year's growth. No. 4580.  $\times 1$ .



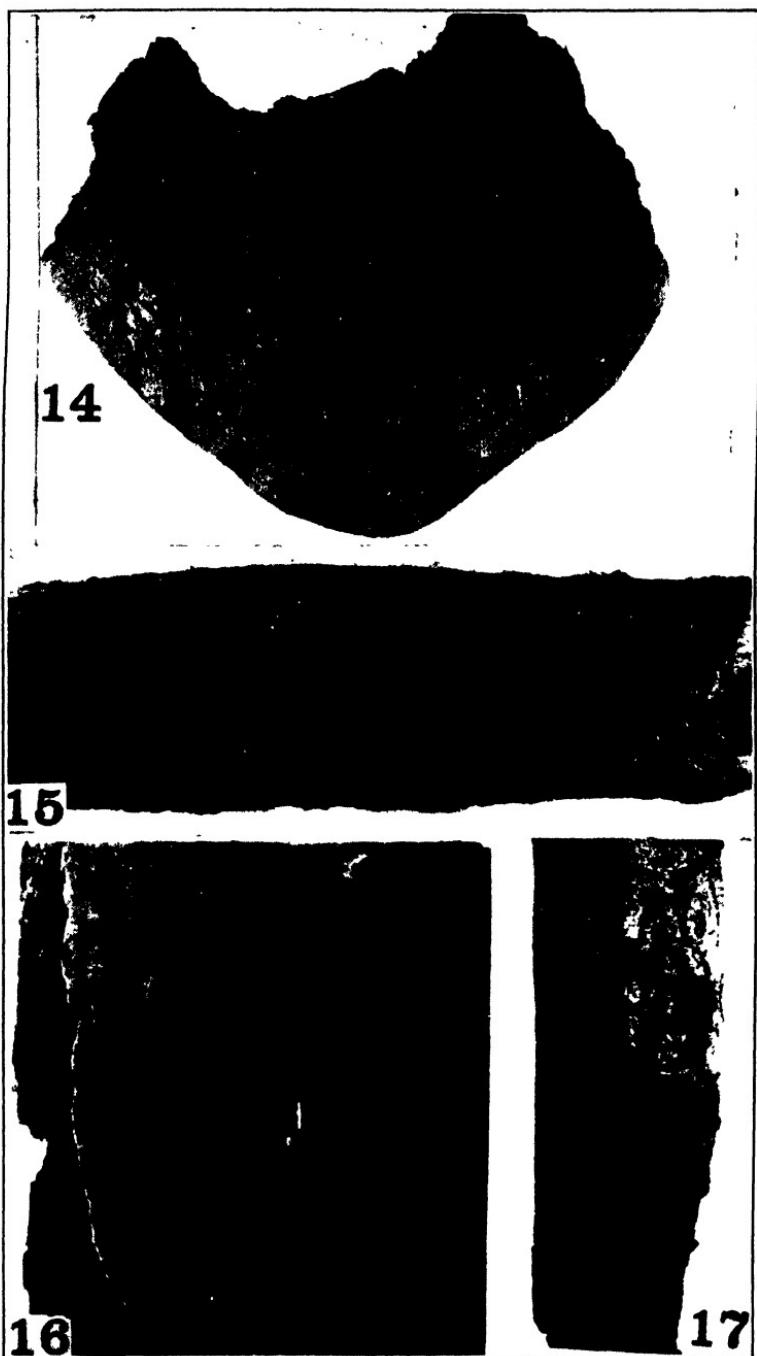
1, 7. *Poria Weirii*; 2, 6. *Poria prunicola*; 3. *Poria punctata*; 4. *Poria tsugina*; 5. *Poria ferrea*.





8. *Poria prunicola*; 9, 11, 13. *Poria Weirii*; 10, 12. *Poria ferrea*.





14, 16. *Poria tsugina*; 15, 17. *Poria punctata*.



Fig. 9. *Poria Weirii*. Photo-micrograph of cross section of tubes, showing the setal hyphae imbedded in the walls of the tubes and some projecting into the lumen of the tubes. No. 3927.  $\times 170$ .

Fig. 10. *Poria ferrea*. Photo-micrograph of cross section of the tubes, showing abundance of setae. Type of *P. fulvida*.  $\times 60$ .

Fig. 11. *Poria Weirii*. Photo-micrograph of cross section of the tubes, showing abundance of setae projecting into the lumen of the tubes. No. 3927.  $\times 150$ .

Fig. 12. *Poria ferrea*. Photo of specimen from L. Romell. No. 11869.  $\times 1$ .

Fig. 13. *Poria Weirii*. Photo-micrograph of small portion of one tube wall in longitudinal section, showing the projecting setae curving out from the trama of the wall. No. 3927.  $\times 170$ .

#### PLATE 14

FIG. 14. *Poria tsugina*. Photo of vertical section through a sporophore 12 to 15 years old, pendant from the lower side of a branch of *Tsuga*. The annual tube layers are fairly distinct. No. 5893.  $\times 1$ .

FIG. 15. *Poria punctata*. Photo of typical specimen. No. 8658.  $\times 1$ .

FIG. 16. *Poria tsugina*. Photo of a thinner specimen from the lower side of a prostrate log. No. 4516.  $\times 1$ .

Fig. 17. *Poria punctata* Fries. Photo of specimen partially cut away to show the very apparent tube layers. No. 9051.  $\times 1$ .

## NOTES ON IOWA SPECIES OF THE GENUS IRPEX

K. CEJP

(WITH 1 TEXT FIGURE)

The genus *Irpex* Fries is one of the most interesting of the genera of the Hymenomycetes, especially with reference to its systematic position. Most mycologists place it in the family Hydnaceae because of the teeth upon which the hymenium is borne. In time, however, these teeth unite in rows and in various comb-like, labyrinthiform patterns until they sometimes assume the appearance of species of *Lenzites* or *Daedalea*. In addition, the general consistency of its hymenium and its microscopic features require that this genus be placed in the family Polyporaceae, as is done by Quélet<sup>1</sup> and by Bourdot and Galzin.<sup>2</sup>

Most of the species referred at the present to this genus were included by Patouillard in the genus *Coriolus*. Some, such as *I. deformis*, *I. obliquus* and *I. paradoxus*, are usually considered as forms of *Poria mucida* Pers. (1).

The largest number of the species of *Irpex* are included in the section Resupinati in which the receptacle is effused on the substratum. Because of their slight morphological difference these species are rather poorly understood. The European species of this section were subjected to a revision by Pilat (2) chiefly on the basis of material from Bohemia collected by Velenovský (5).

In this paper I refer to certain species of the genus *Irpex* from Iowa which I received through the kindness of Mr. G. W. Martin. Some of these species are very rare; some have not as yet been found in Europe. The most abundant species of the Iowa collection was *Irpex lacteus* Fries. Saccardo (4) distinguishes four sections in the genus *Irpex*, according to the presence or absence of a stem and the shape of the hymenium. The species from Iowa are to be referred to his last two sections.

<sup>1</sup> Flore Myc. Fr. 376. 1888.

<sup>2</sup> Bull. Soc. Myc. Fr. 41: 148. 1925.

## SESSILES VEL EFFUSO-REFLEXI MARGINATI

*Irpe x lacteus* Fries, Elench. Fung. 1: 145. 1828.

Effused, at the borders shortly reflexed, cortical, often roof-shaped, concentrically sulcate, villose, white, at the margins byssoid. Teeth arranged in rows, dense, flat, often compressed, thin, sharp, sometimes subdivided, milk-white, later ochraceous. Spores ovoid-globose,  $2 \times 4\text{--}6 \mu$ .

Distribution: North America, Asia (Siberia), Europe (England, France, Russia). In Abiete in Europa etiam in America arctica (Klotsch), ad truncos arborum pr. Minussinsk Siberiae asiaticae, ad ramos et truncos emortuos arborum frondosarum Forestburg, New York, Amer. foeder., ad *Quercus* in Carol., inf. (Saccardo 6: 484). On birch, fir, pine, beech and mountain ash (Rea, 3).

On *Quercus macrocarpa*, West Okoboji, June 15, 1926, Lohman, Longnecker and Martin; on decayed limb of *Quercus*, Iowa City, October 14, 1923, G. W. Martin; Iowa City, September 30, 1923, M. N. Baird and G. W. Martin; West Okoboji, August 17, 1926, G. W. Martin.

*Irpe x hirsutus* Kalchbrenner, Sib. Gomb. 17, t. II, f. 1.

Effused, often imbricated, shaggily villose, white. Teeth fairly large, almost foliated, irregularly arranged, yellowish, never white. Very similar to the species *Irpe x lacteus* Fries. Rare.

Distribution: Hungary, Siberia. On trunks of deciduous trees.

On *Quercus*, Iowa City, October 15, 1923, G. W. Martin.

*Irpe x griseofuscus* Mont. Syll. Crypt. 174. 1856. (Saccardo 6: 487.)

Coriaceous-membranaceous, often semicircular, on the surface semicircularly sulcate, with light or darker stripes; dense, short, grayish-brown, shaggy hair. Teeth either awl-like and short, or labyrinthiformly connected, dense, sometimes almost lamelliformly connected, light brown, white pruinous, at the base reticulately connected. Very rare.

Distribution: Known only from the northern part of South America (Guiana, Leprieur).

On fallen *Acer*, Black Hawk County, Iowa, August 10, 1925, G. W. Prescott; Iowa City, October 6, 1923, G. W. Martin.

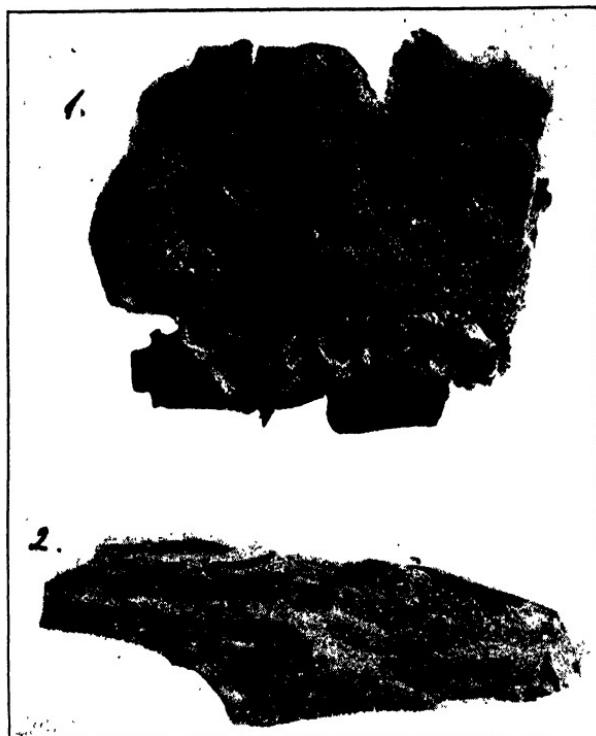


FIG. 1. *Irpex hirsutus* Kalchb. 2. *Irpex obliquus* (Schrad.) Fries.

#### RESUPINATI

*Irpex obliquus* (Schrad.) Fries, Elench. Fung. 1: 147. 1828.

Frequent in Europe and North America (Peck). In Europe frequently collected and described (Fries, Bresadola, Massee, Rea, Quélet, Schroeter, Killermann, Velenovsky, Pilat).

Iowa City, October 6, 1923, G. W. Martin.

*Irpex fimbriaeformis* Berk. & Curt.; Berk. Grevillea 1: 145. 1873.

Totally effused on the substratum, not raised, margin indistinct, pale. Teeth arranged in rows, enlarged at the base, elongated above, yellowish brown. Very rare.

Distribution: Pennsylvania, Amer. foeder., Michener (Sacardo).

On *Quercus*, Homestead, Iowa, May 10, 1925, F. S. Paine.

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# THE ASCIGEROUS STAGE OF COLLETO- TRICHUM LAGENARIUM INDUCED BY ULTRA-VIOLET IRRADIATION

F. L. STEVENS

(WITH 3 TEXT FIGURES)

*Colletotrichum lagenarium* (Pass.) Ellis & Hals.

This was first described by Passerini in 1867<sup>1</sup> as a *Fusarium* and what may have been the same fungus was seen by Berkeley in 1871–76 on various cucurbits.<sup>2</sup>

It was also seen by Roumeguère in 1880<sup>3</sup> who on the advice of Saccardo transferred it to the genus *Gloeosporium*. It was listed in America in 1885.<sup>4</sup> The presence of setae in the acervuli was first noted in 1889 and in 1893 Ellis and Halsted gave to the fungus its present name.<sup>5</sup>

It is now known in Italy, England, France, Australia, America, and, being the cause of a disease of serious economic importance, it has many times been the subject of observation and extensive study. Although sixty-two years have elapsed since the first record of this fungus, during which time many mycologists in many countries have observed, studied and cultured it and always with close observation for the perithecial stage, no one has heretofore succeeded in finding such stage.

Perithecia, however, have been produced abundantly by irradiation<sup>6</sup> with ultra-violet, and only by this means, upon two strains of *C. lagenarium*, one my own isolation from melons

<sup>1</sup> Passerini, G. See Centralbl. Bak. II. 44: 123. 1915.

<sup>2</sup> Berkeley, M. J. Gard. Chron. 1871: 1194. 1871; II. 6: 269. 1876.

<sup>3</sup> Roumeguère, C. Nouvelle apparition en France du *Gloeosporium* (*Fusarium*) *reticulatum* Mt. destructeur des melons. Rev. Myc. 2: 169–172. 1880.

<sup>4</sup> Ellis, J. B., & Everhart, B. N. The North American species of *Gloeosporium*. Jour. Myc. 5: 118. 1885.

<sup>5</sup> Ellis, J. B., & Halsted, B. D. Bull. Torrey Club 20: 246–250. 1893.

<sup>6</sup> For other papers on ultra-violet irradiation by the author, see Science, III. 68: 1923, and Bot. Gaz. 86: 210. 1928.

grown here, the other a strain isolated in Georgia and sent to me by Dr. B. B. Higgins.

The almost universal manner of their appearance is as follows: Very shortly after irradiation, twenty-four hours or less, the entire irradiated region of the colony is considerably darker than that of the non-irradiated region and then or very soon certain

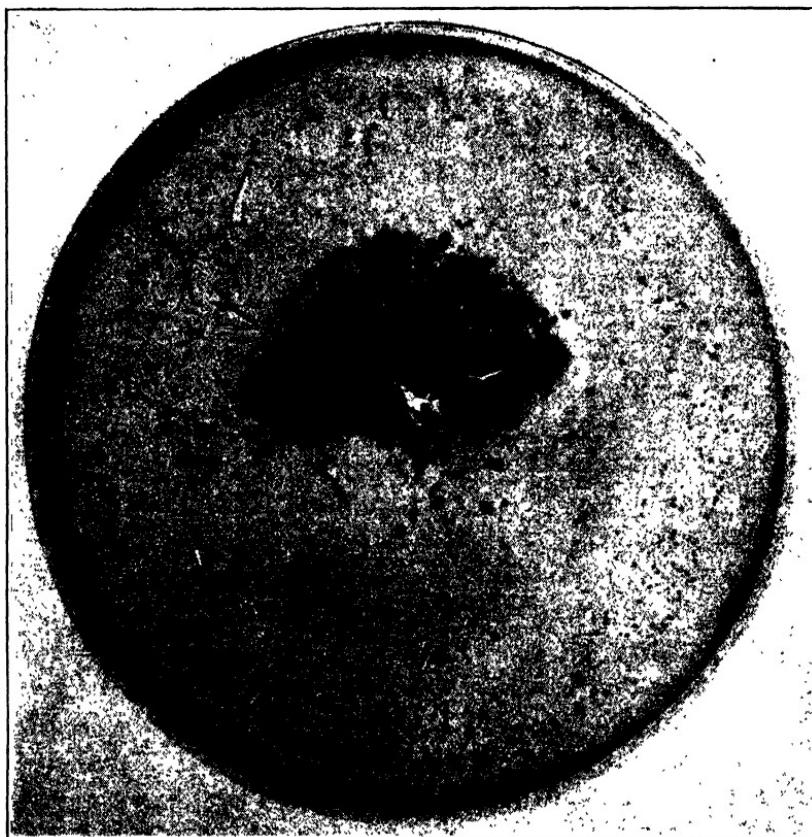


FIG. 1. *C. lagenarium* isolated from melon at Urbana. Many black perithecial plexi shown, immersed on the irradiated half of the plate.

areas stand out as especially dark regions due to large increase in both the density and color of the mycelium in these regions which I shall hereafter refer to as "plexi" (FIG. 1). These are so dense and dark as to be opaque except at the edges. A usual size is  $230\ \mu$  in diameter for the central dense region, or  $800\ \mu$  in

diameter if all the darkened region is included. There is, however, no regularity as to size or density of the plexi. They are sometimes two or three times the diameter indicated above and sometimes even though large may be less dense or dark. Sometimes the dense plexi are evidenced merely by a very slight increase in density and color in a given region. No plexi appear in the nonirradiated area. Later the perithecia increase in number in the plexus though always remaining loosely grouped and with no stromatic development. Still later perithecia may develop in the open space between plexi, but then in no such profusion as when near plexi.

Within a short, 1-2 day, or considerably longer, 5-10 day period after the formation of these plexi, perithecia form in their outer regions (FIG. 2). Not all plexi bear perithecia, but most do so. In their early stages the perithecia are quite like those of *G. cingulata*, at first hyaline, later turning dark and showing reticulations. Though their development follows precisely as does that of *G. cingulata* they never attain a size greater than 110 m. Only immature ascospores were seen.

The young perithecia found on the edges of a plexus are borne on the hyaline mycelium. The perithecia first formed may be regarded as primary and are buried within the agar. The secondary crops of perithecia are superficial. It is quite possible that the primary perithecia are buried because the irradiation killed the superficial mycelium and that the secondary crop is superficial because it is borne on new growth since irradiation.

On one exceptional plate this fungus at one day after irradiation was decidedly and evenly darkened; four days after irradiation it showed numerous perithecia mostly hyaline, but some reticulated and slightly darkened and evenly distributed, not grouped in plexi over the whole of the irradiated area. At twenty days these perithecia did not exceed 55  $\mu$  in diameter and many were still smaller and hyaline. Sometimes the perithecia appear at a much later period even to twenty-three or thirty days associated with the usual special mycelial plexus.

It is quite typical of this species for the sexogenetic influence to extend over into nonirradiated regions; to new growth on the irradiated side or to old growth on the nonirradiated side of the

plate, and there superficial perithecia are formed. Such regions appear first after a considerable interval, even up to fifteen days, as areas of darkened mycelium. Ordinary new mycelial growth beyond the irradiated area is hyaline. Later perithecia develop in them or on them. Not all such dark regions produced perithecia, but all perithecial plexi that are formed in nonirradiated regions are in close proximity with plexi induced by irradiation.

The Georgian strain was tested by irradiation of thirty second repeated five times at intervals of two hours. There was no significant consistent difference in the number of perithecial plexi induced.

Variations in dosage gave the following:

- 1" no perithecial plexi.
- 2" no perithecial plexi.
- 15" 7 perithecial plexi.
- 30" 11 perithecial plexi.
- 60" 6 perithecial plexi.
- 90" 3 perithecial plexi.

In all instances plexi were more abundant in the zone that was one day old when irradiated.

From the study of over 1,000 acervuli of *C. lagenarium* one of my students (Mr. G. H. Boewe) whose results are as yet unpublished states, "data were obtained by studying some 1,000 acervuli and growing some 150 cultures. Of the total number of acervuli examined, there were forty-three more on the nonirradiated side of the colony that produced no setae than on the irradiated side.

"Of the number of acervuli that produced setae, there were more setae per acervulus on the irradiated side of the colony than on the nonirradiated side. There were 760 setae produced by 170 acervuli on the irradiated side as compared with 495 setae by 126 acervuli on the nonirradiated side of the colony. The portion of the colony which was irradiated produced the largest number of acervuli."

Usually, but not always, there are numerous pink acervuli scattered evenly over the nonirradiated growth while but few are on the irradiated area. Occasionally, however, the tips of

the mycelium when irradiated respond with numerous acervuli, thus forming a perfect arc marking the position of the mycelial tips when irradiated. Stronger dosage, however, may suppress them in the irradiated area (FIG. 3).

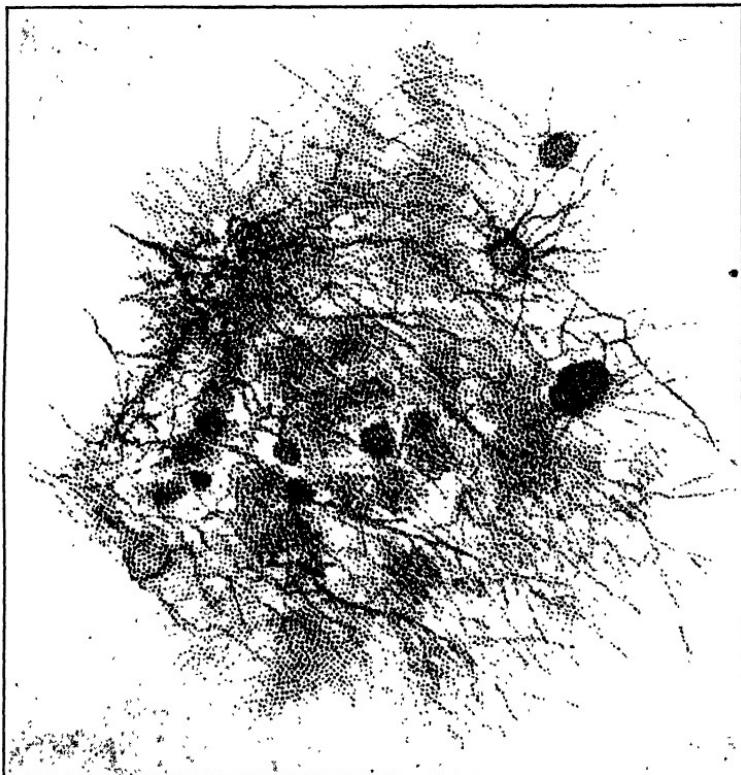


FIG. 2. Plexi of *C. lagenarium* showing perithecia.

Since the ascigerous stage of this fungus is not known and is clearly cogeneric with *Glomerella cingulata* I therefore propose its name as *Glomerella lagenarium* with the following synonymy.

***Glomerella lagenarium* (Pass.) Stevens, comb. nov.**

*Fusarium lagenarium* Passerini, Erb. Critt. Ital. s. 2, No. 148.  
1868

*Gloeosporium lagenarium* (Pass.) Sacc. & Roum. Rev. Myc. 2:  
200-202. 1880.

*Gloeosporium reticulatum* Roumeguère, Rev. Myc. 2: 167-172.  
1880.

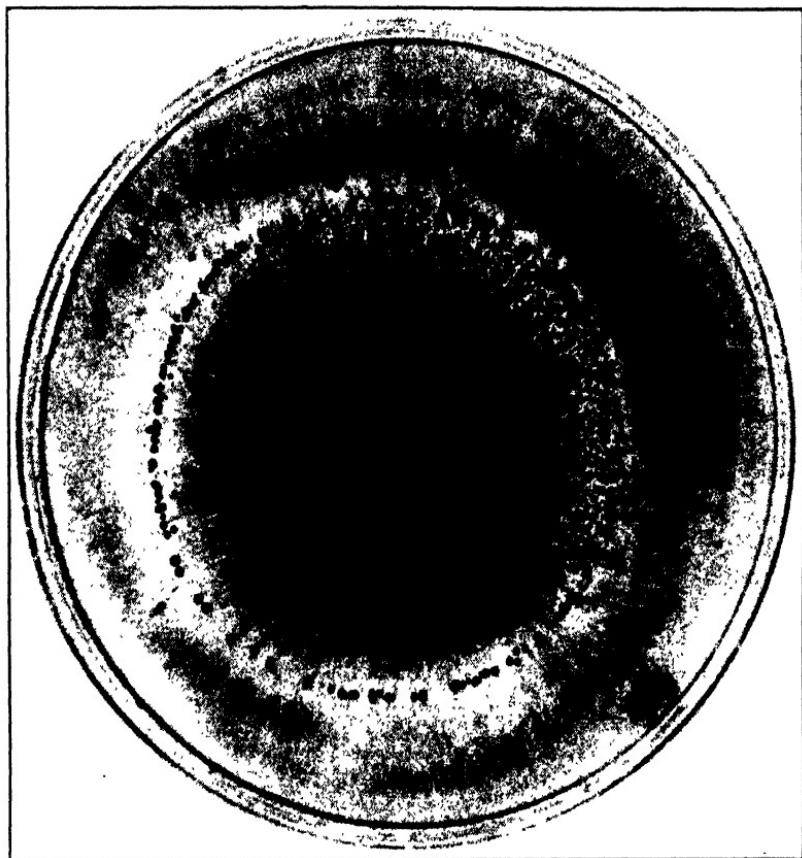


FIG. 3. *Colletotrichum lagenarium* showing a circle of acervuli in the non-irradiated area, none on the irradiated area.

*Colletotrichum oligochaetum* Cavara, Rev. Myc. 11: 191. 1889.  
*Colletotrichum lagenarium* (Pass.) Ellis & Hals. Bull. Torrey Cl. 20: 250. 1893.

The species may be characterized as follows. Perithecia known only as induced on corn meal agar by ultra-violet irradiation, globose, dark, up to 110  $\mu$  in diameter; asci numerous. Spores hyaline, one celled. Conidial form *Colletotrichum lagenarium* (Pass.) Ellis & Hals.

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# CONTRIBUTIONS TO THE CLASSIFICATION OF TORULOPSIDACEAE—I. AN AMERICAN VARIETY OF THE TORULOPSIS MINUTA

R. CIFERRI

(WITH<sup>1</sup> TEXT FIGURE)

## INTRODUCTION

This strain (No. 151 of Ciferri collection) was isolated and partially studied by Starkey and Henrici (7), who discovered it together with yeasts and anascoporic yeasts in soils of Minnesota. The authors, who kindly sent it to the writer for further consideration of its systematic position, classified it under the provisional name of *Torula glutinis* with the following diagnosis: "The young cells are small and oval, and are relatively free from vacuoles and granules. In old culture the cells contain a large fat globule. Agar slant cultures are abundant and very mucoid in character. After a time most of the growth slides to the bottom of the tube. In liquid media some turbidity and an incomplete ring pellicle are produced. No fermentation occurs with any of the sugars."

Ciferri and Redaelli (3), who made a monographic revision of the anascoporic yeasts (Torulopsidaceae) pigmented in red, demonstrated that the *Cryptococcus glutinis* Fresenius, later on denominated *Torula glutinis*, is a specific entity still unknown. As a matter of fact the description is somewhat vague or incomplete; it lacks the essential cultural characteristics and nothing is said about its biochemical activities. In addition to this, there was doubt later on as to whether the *Bacillus prodigiosus* Flügge was included. Therefore *Cryptococcus glutinis* described by Engel under the name *Saccharomyces roseus* is probably different from *C. glutinis* Fresenius, and according to Vuillemin corresponds with *Saccharomyces Freseniusi*. Hansen admits the existence of two different *C. glutinis*, and Schröter confirms the view. As time goes by and further studies are made, a considerable number of strains are described under the name *C.*

*glutinis*, either taking as a basis the color of the colonies or the shape of the gemmate cells. To avoid a long list of authors, reference may be made to the monograph written by Ciferri and Redaelli (3),<sup>1</sup> who state that the species *Cryptococcus (Torula) glutinis* is insufficiently characterized, as it has not been decided yet whether it is a real Saccharomycete or a Torulopsidacea, and that several other species were grouped under the same name. We are therefore endeavoring to ascertain the exact systematic position of the strain of Starkey and Henrici.

#### CHARACTERISTICS OF CULTURES<sup>2</sup>

(In agar of Sabouraud, original formula, pH = 6.4, room temperature 22–32° C.)

Colony abundant, of rapid growth, thick, creamy, uniform, center somewhat thicker than edges, even or slightly irregular. Edges are plain, even or grossly sinuous, sometimes consisting of many small colonies, round or partially confluent. Its color<sup>3</sup>



FIG. 1. Colony 3 days old, on must agar (1/2 nat. size). Colony 7 days old, on Sabouraud agar (1/2 nat. size).

changes from English red (7.R-0.i) to chestnut (9.0R-0.m) and to auburn (11.0.m) when the colony is old.

<sup>1</sup> Also for bibliographical references, refer to report by Ciferri and Redaelli (3).

<sup>2</sup> For methods of study of the Torulopsidaceae and the systematic position of these fungi, refer to Ciferri and Redaelli (4) and Redaelli and Ciferri (5).

<sup>3</sup> The nomenclature of colors is that of Ridgway, "Color Standards and Color Nomenclature." Washington, 1912.

In malt agar (Difco) (pH = 6.4). Colony similar to preceding but of lighter tints.

In agar of Gorodkowa (pH = 6.6). Culture not abundant. Colony of difficult growth, color changeable from shrimp pink (5.00-R.f) to safrano pink (7.R.0.f). Does not present special characteristics.

In must agar. Growth abundant and rapid. Colony similar to preceding, color changing to different shades of grenadine.

In glucosic broth agar (pH = 6.2). Culture entirely similar to that on agar of Sabouraud.

In corn meal agar (Difco) (pH = 7.2). Colony is almost white. Substrate entirely unfavorable; growth of colony slow and difficult.

In prune agar (Difco) (pH = 7.2). Colony of good development, very similar in type and color to that in malt agar.

In potatoes and carrot. Rapid and abundant growth similar in both substrata of culture. The type of colony is always the same; it lacks brilliancy on surface or this takes a porcelain varnish. Color changes from grenadine red (7.R-0) to English red (7.R-0.1) and later on to mahogany red (7.R-0.k).

In malt gelatin (Difco) (pH = 6.6). Colony somewhat rare, viscous and even creamy, at first quite thick and gradually partly liquated; edges indeterminate, central body partly depressed but without crater formation. Color changes from Brazil red (5.00-R.i) to ox-blood red (1.R.k).

Giant colony in must gelatin (pH = 5.8). Colony small, growth not very rapid, almost round, edges irregular, amply lobated, even and thin. The center is depressed and without special characteristics. Its growth is similar to that of the fundamental type I-II of Will. It may be considered as the typical colony of the *Torulopsidaceae*.

In pepto-glucosed water (pH = 6.4). With some difficulty a ring is produced, (which however is incomplete and irregular) but no pellicle appears on surface. The deposit at the bottom of the tube is comparatively abundant, creamy-mucilaginous. The liquid remains clear, but gradually darkens without any peculiar odor. The color of the ring oscillates between orange pink

(11.0.f) and light salmon orange (11.0.d); the deposit oscillates between Sanford's brown (11.0.k) and raw umber (17.0-Y.m).

In must ( $\text{pH} = 5.2$ ). Same as preceding but not so abundant and of lighter hues. The sediment is entirely mucilaginous.

### MORPHOLOGICAL CHARACTERISTICS

In Gorodkowa substrata no ascospores are produced.

In cultures on solid media at laboratory temperature (22–32° C.) the cells are of various shapes, round, oval, elliptical, cylindrical or irregular. The protoplasm, originally hyaline and homogeneous, shows vacuoles in the old cells. The vacuole is generally single and central; only very seldom two vacuoles appear in polar position. The cellular membrane is thin and is only in evidence in old cells. Its dimensions are comparatively constant, varying from 3 to 5  $\mu$  in diameter or length. The giant cells are scarce or altogether absent; they are slightly larger than normal cells.

Cells on liquid cultures do not show marked morphological characteristics or changes. In the deposit at the bottom of the tube the cells are round or almost spherical, about 3 to 4  $\mu$ , occasionally 5  $\mu$  in diameter, and are never catenulate. The protoplasm is not very homogeneous and contains normally one or more crystalloids, with few or no vacuoles. In favorable liquid media, as malt water or grape must, minute chains of 2 to 3 cells are formed, sometimes of even 5 to 6 cells, which may then be smaller in size than on all the other substrata; from 2 to 4  $\mu$ .

The cells of the ring and velum do not differ widely from the preceding; the catenulations, while small, are almost constant; the cellular form is somewhat more elongate. The protoplasm normally contains one or two refractive corpuscles. The giant cells are rare, but present.

### BIOCHEMICAL CHARACTERISTICS

Does not ferment maltose, dextrose, galactose, saccharose, trehalose, lactose, mannose, arabinose, xylose, dulcite, inulin, mannite, dextrine and starch. Assimilates fairly glucose, levulose and saccharose; does not do well in lactose, galactose, inulin and mannose in which it acidifies the liquid.

Assimilates indistinctly tartaric or citric acid; not so well malic and acetic acids; does not assimilate well methyl and ethyl alcohol, but it does well in glycerine.

Assimilates preferably peptone and asparagine quite well; not so well ammonium sulphate and potassium nitrate, but does not assimilate at all potassium nitrite. Liquefies slowly gelatin; generates sparingly hydrogen sulphate from sulphur; reduces actively the methylene blue. Acidifies neutral media and stands one pH = 3.0 in water of autolyzed yeasts, although its multiplication is then very limited. Coagulates milk slowly in which medium it lives well. Does not form tryptophan and indole in pepto-glucosic broth, and does not give the biuret reaction. Does not decompose the trehalose and in the case of the raffinose, if any, the decomposition takes place slowly and with difficulty, but it inverts saccharose generously.

#### SYSTEMATIC POSITION OF THE YEAST

This strain should be classified under the genus *Torulopsis* Berlese emend. Ciferri nec Oudemans. Asporogenous species of yeasts with red pigments, studied wholly with regard to their cultural, morphological and biochemical characteristics, have been found to present similar characteristics with *Torulopsis Montii* Ciferri and Redaelli, distinguished by the great instability of its color and by its low vitality, the thick and numerous giant cells, without marked polymorphism and polymetism and by its assimilative powers. It differs from the *Torulopsis Saitoi* Ciferri and Redaelli by the cells being sometimes larger, by the type and growth of the giant colony, the type of velum produced in liquid media and also by its assimilation, chiefly of nitrogenous substances. It differs from *Torulopsis saccharophoba* Ciferri and Redaelli, and *T. Biourgei* Ciferri and Redaelli, by the absence of cells which are biscuit shaped and elongate, by the giant cells appearing very distinct. It differs from both species by its biochemical characteristics, more especially so from *T. saccharophoba* than from the other *Torulopsis*. The biochemical properties also distinguish the strain in question from *Torulopsis corallina* (Saito) Ciferri and Redaelli (= *Torula corallina* Saito), whose assimilative powers are different and which does not acidify

the liquid culture media. However, this species is quite similar to our strain, as is also *Torulopsis rufula* (Saito) Ciferri and Redaelli (= *Torula rufa* Saito), whose giant colonies differ in shape and color and whose assimilative powers are different. The species which may be regarded closer is *Torulopsis minuta* (Saito) Ciferri and Redaelli, isolated from dust of the air in Manchuria. This species was originally studied by Saito (6), who classified it under the name *Torula minuta*, and was later further investigated, especially with reference to its biochemical activities, by Ciferri and Redaelli (3), who, according to nomenclature adopted by them, classified this species in the genus *Torulopsis*. Its morphological characteristics are the same; the protoplasm of *T. minuta* contains fat corpuscles. As regards the characteristics of cultures, there appears to be no noticeable difference, only slight nuances in color, and in liquid media it forms only the ring. In the biochemical characteristics a few differences are noticed. The affinities between *T. minuta* and our strain are the following: both lack fermentative power; they liquefy gelatin slowly; have no action on starch, cause limited generation of hydrogen sulphate from sulphur; do not form indole in peptonized water; do not assimilate potassium nitrate and only to a slight extent ammonium sulphate; little or no assimilation of ethyl and methyl alcohol; poor assimilation of saccharose.

The differences consist in that the strain in question causes a slight acidity on certain liquid substrata, assimilates well peptone, and fairly well asparagine, inverts saccharose but without acting on the trehalose; assimilates quite well glucose, levulose and saccharose.

The strain under review should be included in the cycle of *Torulopsis minuta*, but owing to its different biochemical activities may be classed in a variety we shall call *americana*.

This is the diagnosis:

*Torulopsis minuta* (Saito) Cif. & Red. var. **americana** Cif.  
var. nov.

= *Cryptococcus glutinis* Starkey & Henrici.

Differs from the type by the presence of giant cells and of small chain-like cells, by the assimilation of peptone and asparagine,

inversion of saccharose but not of trehalose and assimilation of glucose, levulose and saccharose.

Habitat: the soil, in Minnesota, leg. Starkey & Henrici.

It should be noted that the *Torulopsis minuta*, which does not appear to have been found again after its discovery by Saito, is really a cosmopolitan or widespread species, while, owing to its adaptation to new environments, it has changed some of its characteristics. We might mention that, in addition to this North American variety, we have found another variety (var. *parvissima* Cif. & Ashf.) in Porto Rico.

NAT. AGR. STA. AND COLL. OF AGR.,  
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## THE CONCEPT OF MYCORHIZA

ARTHUR PIERSON KELLEY

Fungus-roots, structures which have attracted so much attention in recent years, were probably known to Greeks of the 4th century B.C., since Theophrastus<sup>1</sup> speaks of fungi which grow upon or beside roots of oak as well as of other trees. These fungi, of course, may have been saprophytes or parasites but there is considerable probability that they were mycorrhizal fungi.

A short paper, sometimes stated to have been the first on fungus-roots, was written by Meyen in 1829. It was concerned with the species of *Rafflesia* and *Orobanche*, which he properly considered to be parasites upon other plants. He noted also the short bunches of rootlets formed terminally or laterally on the roots of *Alnus glutinosa*, and called these structures parasites also, considering them to be species similar to *Orobanche* but less highly developed. We now know that the clustered structures are part of the alder roots and that they are primarily infested by bacteria. Other structures described by Meyen from beech roots were possibly true fungus-roots in a strict sense. They underwent, he said, a specific metamorphosis to form short thick branches with pad-like tips, but he considered them to be developmental stages of the parasite *Lathraea*.

The question of the first paper on the subject is of historical interest only, since the first one to draw wide-spread attention to fungus-roots was that of A. B. Frank (1885), in which he told of the occurrence of fungi on the roots of forest trees in Prussia. To the morphological formation of fungus and root which had earlier been called a "fungus-root," Frank gave the name "Mycorrhiza": "Der ganze Körper ist also weder Baumwurzel noch Pilz allein, sondern ähnlich wie der Thallus der Flechten, eine Vereinigung zweier verschiedener Wesen zu einem einheitlichen morphologischen Organ, welches vielleicht passend also

<sup>1</sup>οἱ γὰρ μυκῆτες ἀπὸ τῶν ρίξων καὶ παρὰ τὰς ρίξας φύσμενοι κοινοὶ καὶ ἐπειροῦ εἰσιν.—Enquiry into plants, 3.7.6.

Pilzwurzel, Mycorhiza, bezeichnet werden kann" (l.c., p. 129).

Mycorrhiza, then, is a word spelled originally with one letter r. The word is derived from the Greek words *μύκης* (fungus) and *ῥιζα* (root), and is Latinized in form. Being adopted into the German language, we soon find (at least as early as 1892) a doubling of the letter r to form "Mycorrhiza." This is the usual practice when a prefix is added to a Greek root commencing with r, and has been adopted almost universally by British botanists, and by individual botanists in other countries. In the Germanic languages it was natural to change the letter c to k and thus we find the spellings "Mykorhiza" and "Mykorrhiza" with plurals "Mykorhizen" and "Mykorrhizen." Scandinavians generally use the same spellings. In French the word becomes "mycorhize" (pl. "mycorhizes"), seldom with a doubled r. In Italian the word is "micorize" (pl. "micorizen"). Most American work has been published with the word spelled "mycorhiza" and the plural usually "mycorhizas," although it is sometimes written "mycorhizae." The spelling "mycorhiza" has historical preference<sup>2</sup> while the phonetics of "mycorhizae" are certainly more pleasing than those of "mycorhizas."

Usage of the plural, however, is not uniform, for frequently the singular form of the word is used when obviously it is intended to express plurality.

As originally used the word meant (1) the morphological union of fungus and root (*vide supra*). This is apparently a mutualistic union. Where the fungus is parasitic, the fungus and the parasitized structure form a "pseudo-mycorrhiza," using a term suggested by Melin.

After Frank's publication it was found that exactly similar conditions occur in non-vascular plants, and that fungi live in tissues of mosses and liverworts (see, e.g., Beauverie, 1902). These unions of fungus with tissues not of roots were now called mycorhizae also, and we may speak, for example, of the mycorhiza of a liverwort. The relationship is essentially that of fungu-

<sup>2</sup> Lek (1929) also concluded: "Tegenwoordig wordt, vooral door Engelsche auteurs, het woord veelal "mycorrhiza" gespeld. Daar het feitelijk "mycorhiza" of "mykorrhiza" zou mieten zijn, zijn taalkundig beide spellingen onjuist. Ik hand mij daarom maar aan het woord, zoals Frank (1885) dit invoerde" (l.c., p. 146).

hyphae to individual cells or of a hypha to a cell, and to carry the definition (2) to its logical conclusion we should call the symbiosis (de Baryan definition) of fungus with an alga cell a mycorhiza. No satisfactory term has been proposed for the relationship and the use of the term "mycorhiza" is already well established.

Frequently, especially in popular usage, the fungus associate is spoken of as a mycorhiza, and it is even said that a mycorhiza ramifies through the soil, finally penetrating into the root. This definition (3) finds authority in Sir James Murray's dictionary (1908): "*Myco* (mei-ko), irreg. combining form (for Myceto-) of Gr. *μύκης*, fungus, used in chemical and botanical terms: . . . *Mycorrhiza* (Gr. *ῥίζα*, root), a fungus investing the roots of certain trees and living in close relationship with the surface cells; hence *mycorrhi-zal* a. 1898 tr. *Strasburger's Bot.* 210. Judging from the results of culture experiments made with these plants without mycorrhiza. 1900 *Nature* 28 June 201/2. All known species of mycorrhizal fungi."

It is too obvious to require pointing out that this definition includes only part of the phenomena involved. Nevertheless, the use of definition (3) is common, as when Peyronel (1922) speaks of the "normalle presenza di micorize nel grane e in altre piante coltivee e spontanee"; or Dandeno (1910) states that "mycorhiza shows a step further in adaptation, inasmuch as they attach themselves to the roots . . ." (l.c., p. 35).

The opposite possibility (4), of a root without fungus being called a mycorhiza, finds tangible support in the practice of calling an old mycorhiza by the term after all or practically all of the hyphal material has disappeared from the cells. The cells of such tissues are called, after Magnus, "Verdauungszellen."

Not only true fungi are said to cause mycorhizae, but bacteria as well (5). Thus the nodules found on alder, *Elaeagnus* and other plants, earlier thought to be caused by "Schinzia" but now shown to be due primarily to Rhizobia (or "Rhizobacteria"; Dangeard and Truka, 1929), have been called mycorhizae (cf. Hiltner, 1903).

The word is used further, not to express the existence of a concrete entity, but (6) an abstract idea, the "association" of

fungus and root. This is the definition of Webster's Dictionary (Merriam ed., 1927): "The symbiotic association of a fungus with the roots of a seed plant, as those of the beech and other Fagaceae, those of many heaths (Ericaceae), orchids (Orchidaceae), and most saprophytes." From the literature we might cite Paulson (1924): "The close association of a fungus with the rootlet of a higher plant produces the phenomenon known as mycorrhiza or fungus root, . . ." (l.c., p. 213).

The varied usages of the term are illustrated in the account of "mycorrhiza" found in the Encyclopedia Britannica, fourteenth edition (1929): ". . . *mycorrhiza*, that curious and interesting partnership between the roots of vascular plants and fungus mycelium now known to affect a vast number of the higher plants and to be of great importance in their lives."

Mycorrhizae or fungus-roots ( $\mu\nu\kappa\eta\varsigma$ , a mushroom) ( $\beta\xi\alpha$ , a root) are formed alike by wild and cultivated plants, . . . in 1885, the German botanist, Frank, coined the new name, 'mycorrhiza' (*sic*), . . . ." In this brief account we have the word "mycorhiza" made to mean first (abstract) an association and second (concrete) a structure (fungus-root).

We may summarize the meanings given to the word "mycorhiza":

Concrete: Fungus and root (mutualistic symbiosis)  
Fungus and plant tissue, organ, or body  
Fungus  
Root  
Bacterial nodule

Abstract: "Association" of fungus and root (By inference, "association" of fungus (or bacteria) with plant tissue, organ or body)

Language is naturally subject to development and as continually more ideas are attached to a particular term it becomes necessary to state precisely the exact significance given to the term as it is used. In a strict etymological sense, the word "mycorhiza" means a structure, a fungus-root, and to such a structure the term is most logically applied. In the literature the term is losing its significance and we may find ourselves

under the necessity of inventing new words, each with a more nearly precise meaning.

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## VOLVARIA SPECIOSA

JOHN DEARNES

Mycoprophical authorities are widely divided in their opinion of the edibility of *Volvaria speciosa* Fries; therefore it seems worth while to publish this report of an actual experience.

On Nov. 25, 1927, Mr. E. Davis of Davis Bros., market gardeners, Byron, Ont., brought me a number of fine specimens of a mushroom, which proved to be *Volvaria speciosa*, that he said had appeared spontaneously in the beds in two of their lettuce houses. He was naturally interested in the edibility of the species; so I showed him its published reputation ranging from that of McClatchie, quoted by Lloyd and McIlvaine—"a fine edible agaric and our most abundant one in California"—to that of W. D. Hay and others who label it "poisonous."

A month later, to be exact, on Dec. 27, Mr. Davis brought me a basket of the *Volvaria* for trial as an esculent and told me the story of his experience. He said that they continued to shoot up in abundance in two of the houses—he had counted as many as 66 fine mature plants at one time—and that they looked so clean and attractive that he risked cautiously testing McClatchie's verdict. Finding no ill effects from the trials, he and his family and their friends had been eating them for a couple of weeks or longer.

I used the opportunity of sampling the contents of the basket. The plants could not be in finer condition for the pot. They proved to be edible without a doubt but to my taste their quality was not high. On the basis of 100 for the meadow mushroom at its best these seem to rate about 60. Portions kept over developed a strongly disagreeable odor; possibly the bad reputation of the species is due to eating old plants.

This is a composite description of the finest half-dozen that came into my hands:

*Volvaria speciosa* Fries. Cap white, pale watery yellow at the center, subglobose-bell-shaped at first becoming widely

campanulate and obtusely umbonate, covered with a thin gluey viscosity, drying striate on the margin. Cuticle thin, separable half way to the umbo. Flesh thin, open-grained; odor hardly perceptible to unpleasant; taste rather insipid. The largest campanulate cap 15.8 cm. over the top and covering a circle 12.5 cm. in diameter (4 to 6½ inches); weight 2½ to 3½ ounces.

Gills flesh color to rusty; free from the stem.

Stem reaching 18 cm. in length, slender for its height, not hollow, white, slightly tapering upwards, sheathed for an inch or two at its base by a white, tomentose volva.

Spores rusty-flesh color, large, 16–17 by 7–9 microns, but varying a good deal beyond these limits.

Fruiting abundantly in richly manured sandy loam.

Contradiction of opinion regarding the edibility of this *Volvaria* may be due to confusion of names. Most authors regard *V. gloiocephala* DC. as poisonous. P. Dumee (1909) declares that the last name belongs to *V. speciosa* Fries and accordingly transposes the two names. Rea in British Basidiomycetes (1922) lists *V. gloiocephala* (DC.) Fries as poisonous and cites Maire as re-putting *V. speciosa* Fries to be edible. Maublanc (1926) makes *V. speciosa* Fries a synonym of the other name and states that the investigations of Chauvin and Gauthier have proven it—or both—to be edible.

LONDON, ONT.

# THE OCCURRENCE OF SCHIZOPHYLLUM COMMUNE ON GREEN APPLES

F. D. BAILEY & S. M. ZELLER

(WITH 1 TEXT FIGURE)

We have just read with interest the report of the infection of dormant sweet potatoes by *Schizophyllum commune*.<sup>1</sup> This is an interesting additional type of host for this fungus. In this connection the authors thought the occurrence of this fungus on green apples worth a note. In the fall of 1926 in an orchard in



FIG. 1. Apples with sporophores of *Schizophyllum commune*.

Marion County, Oregon, the green apples thinned from the trees during the summer literally covered the ground under the trees. As a rule the upper surface of many of these apples was sunburned and shriveled but otherwise they were well preserved. In a high percentage of cases, however, these apples under Spitzenberg and Red Cheek Pippin trees were infected with *Schizophyllum commune*. The affected tissue was a brown dry rot. Any cavities in the tissues were filled with fungous felt and in many cases the apples were covered with this white felt. By October

<sup>1</sup> Poole, R. F. Sweet potatoes infected by *Schizophyllum commune*. Jour. Elisha Mitchell Sci. Soc. **45**: 137-139. illus. 1929. (Rev. Appl. Myc. **9**: 267. 1930.)

and November these apples had taken on wings in the form of mature sporophores. These originate at most any point on the surface of the apple or stem. Two of our many illustrations are presented herewith (FIG. 1).

OREGON STATE COLLEGE,  
CORVALLIS, OREGON

## NOTES AND BRIEF ARTICLES

Doctors B. O. Dodge and Fred J. Seaver attended the Annual Meeting of the American Association for the Advancement of Science as representatives of The New York Botanical Garden in mycology and pathology.

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Dr. L. O. Overholts of the Pennsylvania State College instead of attending the meeting of the American Association for the Advancement of Science at Cleveland spent several days at the Garden studying over collections of Polyporaceae. He was missed at the meeting where he has been very regular in his attendance during past years.

### THE CLEVELAND MEETING

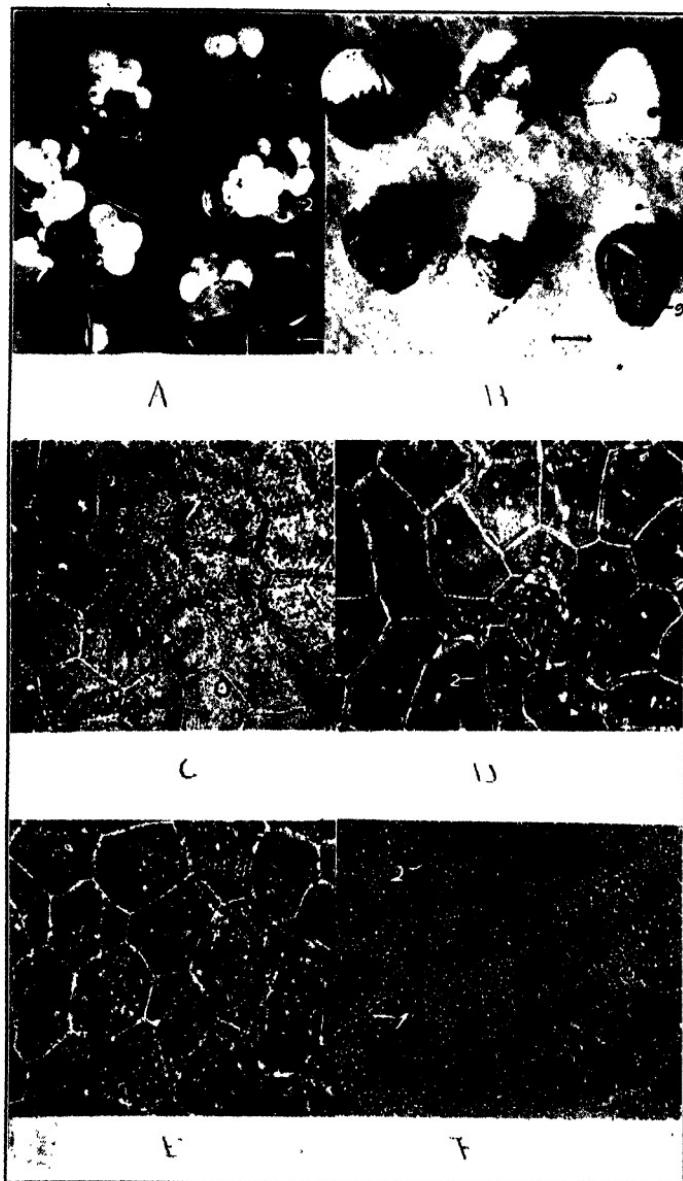
The meetings of the Mycological Section of the Botanical Society of America were held at Cleveland this winter in connection with the American Association for the Advancement of Science as usual. The first meeting of the mycologists was held on Tuesday morning at ten o'clock in the geological building of Western Reserve University. The attendance was about sixty and rather larger than usual. Ten papers were scheduled for the first meeting but three of these were not presented owing to the absence of those scheduled to take part, so that the first meeting closed more promptly than usual.

Wednesday morning a joint session of the Mycological Section and the American Phytopathological Society was held. This was a de Bary program and throughout most interesting. Dr. W. H. Weston of Harvard University gave a very vivid account of the early life and work of de Bary laying special emphasis on the fact that it was de Bary's personality as much as his work which impressed itself upon the hearts of his students. Dr. L. R. Jones of the University of Wisconsin gave a discussion on "The Concept of Parasitism, a Development of de Bary's Early Works." Following this Dr. J. C. Arthur of Purdue University

discussed the problem of heteroecism emphasizing the fact that heteroecism was not first discovered by de Bary although he was one of the first to use the culture method in working out the life histories of the rusts. It was this phase of de Bary's work which impelled Dr. Arthur to take up this line of investigation in which he has contributed much to our knowledge of the rusts. Dr. D. Reddick of Cornell University spoke on "Die Kartoffelkrankheit" and Dr. G. W. Martin of the University of Iowa on de Bary's work so far as it affected "Die Myzetozoen."

The last meeting of the Mycological Section was held on Thursday morning, January 1, the attendance being slightly smaller than that of the first meeting owing to the fact that some had already left for their homes. A number of interesting papers were presented and lively discussion ensued.

In addition to the regular mycological programs, Dr. B. O. Dodge of The New York Botanical Garden, by a special invitation presented his work on "Hybridization and Inheritance in Ascomycetes." The paper as presented was received with much interest on the part of those who heard it. The results of this work were published in the January–February issue of *Mycologia*.



ANTHRACNOSIS OF THE SNOWBERRY

# MYCOLOGIA

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## ANTHRACNOSE, ALTERNARIOSE AND BOTRYTIS ROT OF THE SNOWBERRY

### PART I

#### ANTHRACNOSE OF THE SNOWBERRY CAUSED BY *GLOMERELLA RUFOMACULANS*

W. H. DAVIS

(WITH PLATES 15-19 AND 5 TEXT FIGURES)

During the autumns of 1926, 1927, and 1928, fruits on the snowberry shrub (*Symporicarpos albus* L.) Blake var. *laevigatus* (Fernald) Blake turned a brown color and dropped prematurely. At first, it was thought that this discoloration was due to a freezing of the fruits (snowberries) before they had matured. However, in the autumn of 1928, a careful diagnosis revealed sporulating acervuli of a *Gloeosporium* parasitizing several diseased snowberries (PLATE 15, A and B).

Stewart (1910) reported a *Gloeosporium* which parasitized snowberries but he did not assign a species to the organism. Barrus and Horsfall (1928) stated that they isolated a *Gloeosporium* from the snowberry but were unable to obtain infection when they sprayed the fruits with conidia. From this brief review of the available literature, it appeared an investigation should be undertaken to solve the following problems:

1. How prevalent and widely disseminated is this disease?
2. Is the disease caused by a *Gloeosporium*?

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3. If so, what Latin binomial should be assigned to the fungus in its perfect and imperfect stages?
4. What is the life history of the organism?
5. What fruits other than the snowberry, if any, are parasitized?
6. From a study of the life history of the fungus and development of the disease, what controls could be practiced?

#### SYMPTOMS AND SEASONAL OCCURRENCE OF THE CONIDIAL STAGE

In 1928, several fruits infected with anthracnose were first observed during the month of September but during the latter part of October an average of sixty per cent of the fruits on all shrubs examined were infected. The initial symptoms appeared as minute hazel-colored areas which continued to enlarge until the whole snowberry was a cinnamon-rufous color. Later, these infected snowberries turned shades of hazel but when incubated for 10 days under favorable conditions, they were dotted with small, flesh-colored flecks which were conidial masses or acervuli rupturing the epidermis. Finally, these acervuli turned an olive black or dead black, which was due to an outgrowth and change in color of the mycelium (PLATE 15, FIGS. A AND B, PLATE 16, FIG. A, PLATE 19, FIG. B, 7, 8, 9). Most of the snowberries infected with the *Gloeosporium* shriveled and dropped to the ground or were easily beaten from the shrubs by wind, rain and snow. After detached snowberries had remained on the ground some time, most of them turned black and mummified (PLATE 18, FIG. B, PLATE 19, FIG. B, 9).

However, some of the fruits were parasitized by *Penicillium* sp., *Alternaria* sp., and *Botrytis* sp., and then the snowberries were of a steel gray, a chamois or a yellowish tinge, which was often accompanied by a wet rot (PLATE 18, FIG. A, 3, 4, 7, 8, and PLATE 19, FIG. B).

In April 1929, thirty-six infected snowberries which had been buried under leaves at the base of shrubs and overwintered there during 1928-1929 were brought to the laboratory and placed in a moist chamber. Numerous acervuli bearing viable conidia were observed on eight of them. Furthermore, three weeks later, acervuli bearing viable conidia were also observed on snowberries

which had not been placed in a moist chamber but had remained in their natural environment; on shrubs, in the open.

The flesh-colored spore masses or acervuli often appeared near the veins and on folded surfaces of leaves not exposed to the open air. Excised leaves and fruits which had been inoculated and afterwards incubated in a moist chamber showed symptoms of the disease within eight to eighteen days. These infected areas on the leaf bore a dark or black color as if stained by a dye.

The bark of infected twigs possessed a water-soaked, dark appearance which was ruptured in various places by sporulating acervuli. Here the spore masses appeared flesh-colored as on fruits and leaves. On the shrubs under observation, the tips of these infected twigs did not remain alive during the following season, but generally died back about two or three nodes from the infected inflorescence. These infected tips of twigs did not produce viable conidia of a *Gloeosporium* during the following season when they were dead (TEXT FIGURE 2, A, 1; B, 0; C, 1).

Cankers seldom formed on the stems of the plants under observation. In 1928, no cankers could be located, but in 1929 several cankers were observed on the current season's wood. These occurred near the middle of the internodes, causing a swelling and enlargement in diameter (1.5 to 2 times), presenting the appearance of a healing wound. The wood of the canker was darker in color, cracked lengthwise of the stem and, in October, bore sporulating acervuli with viable conidia around the margins of the rift. In one case there were fruiting bodies of the same size as those of *Glomerella* perithecia but no asci could be found within them as they had reached maturity and were disintegrating.

These observations showed that symptoms of the disease were not in evidence during the blossoming period of July 1928 and 1929 but first appeared on the snowberries in the autumn or about September first. The fungus overwintered in snowberries which had been buried under organic materials and sporulated during the following spring, producing viable conidia; the ascogenous stage was also located in diseased snowberries which had remained outdoors under natural conditions. Furthermore, the first snowberries infected, those at the base of the fruit cluster,

sometimes bore perithecia while on the shrub. Specimens were placed in the Cryptogamic Herbarium of the Massachusetts Agricultural College under the accession number 3155 (PLATE 18, C).

#### GEOGRAPHICAL DISTRIBUTION AND ECONOMIC IMPORTANCE

In 1928, diseased snowberries were observed on each snowberry shrub examined. The disease was widely distributed in Massachusetts, Connecticut, New Hampshire, Vermont, and New York State. Pathologists reported its presence in Iowa, Illinois, Wisconsin, Michigan, Ohio, New Jersey and Pennsylvania.<sup>1</sup> So it would seem that in 1928 the disease was prevalent in northeastern United States. In 1929 the disease reoccurred and was reported prevalent in Vermont, Massachusetts, Connecticut, and New Hampshire.

The snowberry shrub is extensively used in landscaping around dwellings, in gardens, private estates and parks. Its principal beauty lies in the abundance of beautiful, snowy-white fruits from which it derived its common name, snowberry. The shrub is symmetrical, ornamental with and without foliage, is easily cultivated and highly prized by landscape gardeners and others for plantings on estates and for ornamentation in general. The fungus robs the plants of a greater portion of their beauty, as it discolors the leaves and snowberries, causing them to drop prematurely; infects and kills some of the young wood, thereby rendering the plants unhealthy as well as unsightly. Furthermore, the fungus killed seeds which could have been used for propagative purposes. If the fungus should continue to render the fruits as unsightly as it did in 1927, 1928 and 1929, the shrub would probably lose more than half of its economic value as an ornamental in northeastern United States.

#### ISOLATION, CULTURING AND INOCULATION WITH THE PATHOGEN

The fungus was isolated by four methods:

1. Transfer of mycelium. Diseased snowberries were incubated in moist chambers until mycelium of the fungus appeared

<sup>1</sup> Pathologists stated in personal conversation that they believed they had seen the disease on snowberries cultivated in several of the states mentioned.

on their surfaces; then portions of this mycelium were transferred to tubes of potato-dextrose agar.

2. Monosporous culturing. Employing bacteriological methods, spore dilutions were prepared and poured on agar. When a single conidium was observed germinating in a clear microscopic field, it was transferred, on a block of agar, to a tube of potato-dextrose agar.

3. Spore streaking. A sterile needle was dipped in an abundantly sporulating acervulus and the conidia transferred, to tubes of slanted, potato-dextrose agar, by drawing the needle along its surface.

4. Tissue culturing. The surfaces of diseased snowberries and leaves were sterilized, small portions of diseased tissue together with some of the adjoining healthy tissue were removed under aseptic conditions and transferred to tubes of slanted potato-dextrose agar.

By each method, pure cultures of the fungus were readily procured. After incubating a transfer culture for 5 to 10 days at 20° C., the mycelium, which at first was a light-gray, turned to a shade of brown and at the center of the culture showed a black area. Often, the cultures had a tinge of olive-green when observed from the under side. The culture also bore the characteristic flesh-colored spore masses with conidia on acervuli.

To establish the pathogenicity, physiology and host range of the fungus, various fruits were inoculated by spraying their punctured and unpunctured, sterile surfaces with conidia from pure cultures and from sporulating snowberries. Mycelium was also laid on sterile surfaces and inserted under the epidermis of the fruits. Then the inoculated fruits were incubated in Coplin jars lined with dampened filter paper and stored at 20° C. A list of the fruits inoculated follows:

- |              |              |
|--------------|--------------|
| 1. Snowberry | 6. Tomato    |
| 2. Quince    | 7. Banana    |
| 3. Pear      | 8. Cranberry |
| 4. Apple     | 9. Peppers   |
| Baldwin      | Green        |
| McIntosh     | Red          |

5. Grapes	10. Indian currant ( <i>Syphori-</i>
Niagara	<i>carpos orbiculatus</i>
Tokay	Moench)

Leaves and stems of the snowberry were also inoculated and checks prepared for each inoculation made.

Infection was observed in each fruit inoculated except the cranberry, which together with the checks remained healthy. Furthermore, sporulation occurred on each of the surfaces but most abundantly on the quince, apple and banana. The development of the infection areas on the green pepper was very slow, sporulation not being observed until the third week following inoculation, while spores were observed on the snowberries 6 days and on other fruits 5 to 12 days after inoculation. When conidia were spread on the snowberry, apple, pear, and banana, three fourths of the inoculated areas showed infection; on the other susceptible hosts, about one third of the inoculations showed infection. Infection was also obtained when mycelium was placed in punctures of the susceptible hosts.

Excised leaves were inoculated by Clinton's method (2) but were very slow to show sporulation, which occurred either the second or third week following the inoculation. On leaves, the fungus behaved more like a facultative saprophyte than a true parasite. It seemed to sporulate best when the leaves were partly decayed.

At the present time, it is too early to predict the results of the twig inoculations.

#### PATHOLOGICAL ANATOMY

Stained sections on prepared slides showed that the surface of the snowberry is covered by a cuticularized layer varying from three to eight microns in thickness. Under this layer of cutin is the epidermis, which consists of a layer of polyhedral cells with comparatively thick walls which average six microns in thickness (TEXT FIGURE 1, 16, and PLATE 15, FIGS. C, D, E). The walls of the epidermal cells are suberized and this manifests itself as small biscuit-shaped masses of suberin about as thick as the cell wall itself (PLATE 15, FIG. F). In old snowberries, these suberin

thickenings were fastened to each inner wall of the epidermal cells. As seen through the microscope, this suberized material gave the cell wall a rough appearance and probably has much to

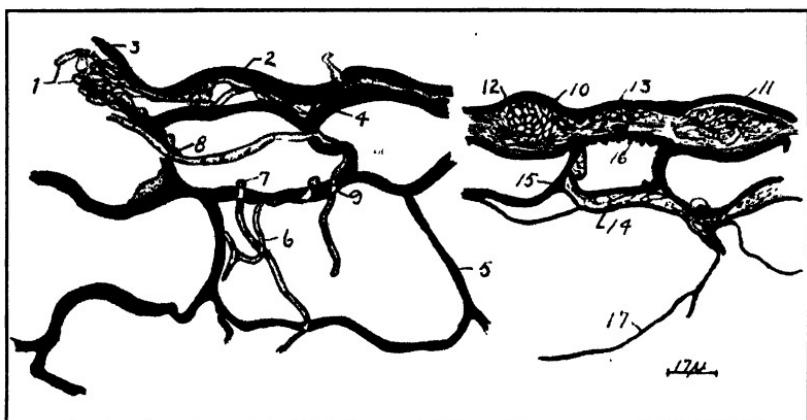


FIG. 1. Drawings of the outer cells in a diseased snowberry showing the position of the hyphae, beginning of an acervulus and penetration of the cell walls by hyphae of *Gloeosporium rufomaculans* collected in November. (Traced by aid of a camera lucida from triple stained sections permanently mounted. numbers 1 to 9 are from one specimen and 10 to 17 from another). No. 1, 2, 11; Hyphae between cutin and epidermal cell walls; No. 3, 10. Cutinous layer. No. 4, 15. Walls of epidermal cells. The pits are omitted; No. 5, 17. Walls of mesocarpal cells; No. 6. Infracellular mycelium of the mesocarp; No. 7; Haustorium; No. 8. Hypha entering through a pit; No. 9. Hypha penetrating the cell wall where no pit could be detected; No. 12. Early formation of an acervulus; No. 13. Deliquescent hyphae; No. 14. Hyphae between epidermal and mesocarpal cells; No. 16. Suberized membrane of an epidermal cell.

do with the snow-white appearance of the snowberry as the material itself is a clear, white color. It was insoluble in concentrated sulfuric acid but soluble in an aqueous solution of potassium hydroxide acting for 4 to 12 hours. It did not turn blue but brown when submerged in a solution of potassium iodide. The suberin was stained green by a hot alcoholic solution of chlorophyll<sup>2</sup> acting for one hour, while the cellulose of the cell walls remained hyaline. The suberin material was drawn from

<sup>2</sup> The chlorophyll solution was prepared by adding, to 300 cc. of 95 per cent alcohol, 75 grams of tomato leaves removed at noon on a clear day. The preparation was boiled gently for 15 minutes, 225 cc. of the solution was decanted and thin sections of epidermal cells of the snowberry were submerged in this solution and placed in a dark chamber for one hour.

the cell wall toward the center of the cell by plasmolysis with glycerin, which shows it is a suberized protoplasmic membrane appearing as a part of the cell wall. Furthermore, this suberin appeared to be laid down in these cells about the time the snowberry changes from its green color, due to chloroplastids in the mesocarp, to the white waxy color. A layer of suberin appeared to be deposited within the upper walls of the epidermal cells (TEXT FIGURE 1, 16).

Under the epidermal layer is the mesocarp, which is composed, for the most part, of many layers of very large cells with thin, smooth, hyaline walls averaging three microns in thickness (TEXT FIGURE 1, 5, 17).

Scattered within the mesocarp are the fibro-vascular bundles and near the center are two seeds in large chambers.

The hyphae and germ tubes from conidia of the fungus penetrated the cuticle or passed unobstructed through stomata into the snowberry (PLATE 15, FIG. D) and multiplied between cuticle and epidermal cells, forming mats of sclerotia which later produced conidiophores and conidia which comprised the acervuli. The rufous color manifested in the external symptoms of the disease is believed to be due to the color of these maturing hyphae as seen through the cuticle. However, when the mycelium turns black, the old infected snowberries also appear black (PLATE 16, FIG. A, 6, 12, 19). Later, the hyphae pass from cell to cell by dissolving the suberin or passing through pits in the epidermal cell walls. Finally the hyphae reach the mesocarp and the central chamber containing the seeds (TEXT FIGURE 1, NUMBERS 11, 12; PLATE 19, A).

The initial infection of snowberries in the autumn would seem to be produced by hyphae coming from infected stems and flower stalks through pedicels into the berries and thus arriving at the mesocarp before reaching epidermal cells.

Observations in 1928 showed that the disease first appeared in the autumn, September, and that in most cases the oldest snowberries or the ones nearest to the main stems were first to show symptoms of anthracnose. Furthermore, twigs bearing diseased fruits died back from the tips a distance of two to three nodes. These observations seemed to furnish evidence of systemic,

perennial mycelium. An attempt was made to detect the fungus within the twigs and trace it to its destination. In July and August, 1929, young twigs were hand-sectioned and the sections mounted in green lactophenol. These twigs had grown from 1928 twigs which had borne diseased snowberries.

In July, the pith cells in the tips of the young twigs were yet filled with starch grains while those next to the old parent stem were somewhat disintegrated, as the starch had been removed. Here, hyphae of a fungus were present in abundance. A *Gloeosporium* was cultured from sections of those stems which had been externally sterilized and incubated on potato-dextrose agar. Later, hyphae could be detected in the medullary rays of the wood.

To determine the presence of this fungus within stems, the exterior of four stems bearing diseased snowberries was sterilized by submerging them for 10 minutes in an aqueous solution of bichloride of mercury 1 : 1000 with the addition of 2 cc. of HCl and then rinsed in sterile distilled water. Sections were removed under aseptic conditions and incubated on potato-dextrose agar (TEXT FIGURE 2).

The results may be summarized:

1. The dead wood at the tips did not contain a viable *Gloeosporium* but near the lower node (TEXT FIGURE 2, *A*, NUMBERS 1, 2) two sections showed the presence of a viable *Gloeosporium*.
2. Most of the internodes contained hyphae of a *Gloeosporium* (*A* and *B*, 7, 10, 11, 12, 13, 20, 24, 25, 26); the cultures from *C* (6, 11, 12, 13) did not sporulate well save 11, but possessed the characteristic mycelium. However, the cultures contained more actively growing mycelium and sporulated best from the nodes and from older wood.
3. Most of the twigs of the current year's growth contained hyphae of the *Gloeosporium* from base to tip. An exception was that shown in Text figure 2, *B*, 41 to 45. Here, the mycelium did not sporulate except in 44, which showed a *Gloeosporium*. In twig *C*, sporulation around sections incubated from the lower twigs was poor or none at all (NUMBERS 9, 10, 14, 15).
4. Eighty per cent of the autumn buds contained hyphae of a *Gloeosporium*. Buds were removed from branches containing

infected berries (*D*, 5, 6, 8) and one from each of eight nodes on a branch bearing apparently healthy berries (*D*, 7). Buds from each of these twigs contained *Gloeosporium* mycelium most of which sporulated when cultured.



FIG. 2. Diagrams showing where sections were removed from sterilized strawberry twigs. These sections were incubated on agar to detect *Glomerella rufomaculans*, if present, within the tissues during September and October, 1929. Numbered triangles denote the parts cultured; diseased fruits and ovaries are shown in black. A. A branch with dead twigs of 1928 (1, 2) and live twigs bearing diseased snowberries (5, 6, 9); B. Similar to A, only collected two weeks later. The twigs on this branch bore diseased fruit, save 46 and 47. Sections 21 to 23 were removed to determine whether mycelium of *Gloeosporium rufomaculans* could be present in a twig and not apparently infect the fruit; C. Similar to B, a check; D. Buds were removed from these twigs (5, 6, 8) bearing diseased berries; also, from 7, which bore apparently healthy berries. Culturing methods showed a *Gloeosporium* present in most of them.

5. Flower stalks (floral axles) and pedicels from the tips of three twigs with diseased snowberries were externally sterilized and incubated on agar. A *Gloeosporium* was evident in each culture.

6. Hand sections showed hyphae within the outer bark of twigs and between the outer bud scales within the buds.

From the above observations and data, it would seem that

initial infections generally occur in bud scales or the bark. From these parts, the hyphae penetrated the pith cells of the new growth or occupied the hollow cavity of the maturing stem from which they entered the flower stalk and pedicel into the berry. Conditions are not suitable for the passing of hyphae from the stem into the fruit earlier in the season than September. Furthermore, hyphae can occupy an area around the lenticels. It was not decided whether hyphae from the pith could penetrate the medullary rays and enter the bark, but the writer believes they can, as hyphae at one node seemed to do so. This being true, the fungus might advance from old growth into new.

In the twigs, the acervuli were mostly confined to the current year's growth. Diseased twigs bearing acervuli were collected in December and free-hand sections made and mounted in lactophenol containing acid green. By this method, the hyphae were well differentiated from the wood and bark elements, for they were stained a brilliant green. In these sections, the hyphae were most evident just beneath the epidermis; sometimes occupying areas from the outer corky bark cells to the cambium. Many of the cells were hypertrophied and filled with the sclerotial masses which had ruptured the outer layers of bark and formed sporulating acervuli. These acervuli appeared black at the ends but in sections of acervuli sporulating on twigs which had not been removed from moist chambers the spore masses were sometimes a flesh-color which is characteristic of this *Gloeosporium*.

Acervuli ruptured the epidermis, appeared black, were scattered but not numerous. The tips of infected twigs soon died, together with their winter buds. However, buds on apparently healthy wood directly below the dead tips of twigs formed new growth.

Permanent, stained mounts were made from microtome sections of infected leaves of the snowberry plants. In the sections examined, the hyphae penetrated the palisade and mesophyll cells but were most numerous around epidermal cells. The hyphae formed sclerotial masses which were the sources of acervuli (TEXT FIGURE 3, NUMBERS 7, 13, 14). In the leaves examined, every tissue except the xylem was penetrated by hyphae of the *Gloeosporium*.

## CONIDIAL STAGE OF THE PATHOGEN

Free-hand sections of dried, diseased strawberries were mounted in green lactophenol and 20 cells of the parasitic mycelium

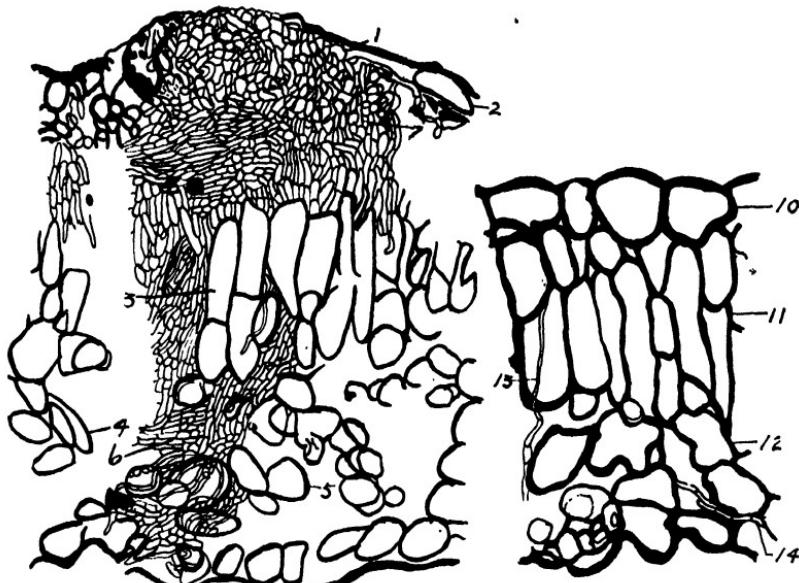


FIG. 3. Outline drawings showing cross-sections of a diseased strawberry leaf collected in October. (Tracings by aid of a camera lucida, from triple stained sections permanently mounted; numbers 1 to 6 were near the midrib and 10 to 14, two millimeters nearer the margin of the same leaf). No. 1. Cutin on the upper surface; No. 2, 10. Epidermal cell; No. 3, 11. Palisade cell; No. 4, 5, 12. Mesophyll cell; No. 6, 14. Hyphae in the air spaces between mesophyll cells; No. 7. Beginning of an acervulus, the hyphae have raised the cutin and epidermal layers; No. 13. Hyphae penetrating a palisade cell.

measured; the lengths varied from 17 to 30 microns; diameter, 1.5 to 2.5 microns. Sometimes several hyphae coalesced and under certain conditions they deliquesced. Colors of hyphae in culture: at first, light-gray, finally a dark olive-black to black. Very often a reddish hue appeared in rapid growing cultures. No clamp connections were observed.

The acervuli averaged 101 microns in width; 58 microns deep; often several coalesced. The conidiophores were constricted at the apex where conidia were abstricted; they averaged  $2 \times 20$  microns (PLATE 16, FIGS. B, C). Masses of fresh conidia were pinkish (varying from a flesh-color to a hazel) but individual

conidia were mostly hyaline when observed with a microscope or sometimes bore a wash of brown when old; 100 fresh conidia were collected from several different hosts two weeks after inoculation and the conidia measured (PLATE 16, FIG. D).

A tabulation of standards and limits of variation in the sizes of conidia for *Gloeosporium rufomaculans* obtained from different cultures and hosts. Sizes reported by other investigators are also listed for comparisons.

Sources of Conidia Agar cultures (Snowberry)	Standard in Microns	Limits of Variation in Microns
From conidia . . . . .	5.4 X 15	4.5-7.2 X 12.6-20 <sup>a</sup>
From ascospores . . . . .	5.4 X 20	5.4-6.3 X 12.6-28.8
Banana . . . . .	5 X 19.4	
Apple . . . . .	5 X 15.4	
Tomato . . . . .	5.7 X 15.4	
Quince . . . . .	5.8 X 15.0	
Grapes—Tokay, Niagara . . . . .	5.7 X 15.5	
Stewart's <i>Gloeosporium</i> sp. . . . .	4 X 6-8	
Saccardo ( <i>G. fructigenum</i> Berk.) . . . . .	5-6 X 20-30	
Spaulding, and von Schrenk ( <i>G. rufo-</i> <i>maculans</i> ) . . . . .	4-5 X 12-16	3.5-7 X 10-28
Craig ( <i>G. fructigenum</i> ) . . . . .		5-7 X 10-20

From the above list of measurements, it is to be noted that conidia from a culture transferred from germinating ascospores were longer than those from a monosporous culture from a conidium. Furthermore, conidia on bananas were noticeably longer than those from the other hosts. The conidia from the snowberry seemed to be wider and much longer than those reported by Stewart but compare favorably with those from the apple reported by Spaulding and von Schrenk.

From the preceding description of the pathogen it is to be noted that the fungus is a *Gloeosporium*, as the "spores ooze out in pink masses" (Stoneman), and the acervuli, conidiophores and conidia are characteristic of the genus and the mycelium possesses the culturing characteristics of *Gloeosporium rufomaculans* (Berk.) Thüm. This is a form-species which parasitizes similar hosts, as shown by the inoculations performed, and the morphology described is within reasonable limits, for the fungus parasitizing the snowberry.

<sup>a</sup> When conidia from snowberries were dried for 3 weeks, then mounted in water, they measured 2.8-6 X 9-18.5  $\mu$ . Standard (5 X 12.6  $\mu$ ).

### THE ASCOGENOUS STAGE

The surfaces of snowberries were sterilized, inoculated with conidia from a pure culture, stored in sterilized Coplin jars containing wet filter paper and incubated under aseptic conditions at 20° C. Also, agar plate cultures and snowberries inoculated as described elsewhere in this article were incubated at the same time and under the same conditions.

After these inoculated snowberries had incubated for 4 to 6 weeks, perithecia formed on their surfaces. Perithecia also formed in the agar plate cultures after they had been incubated 8 weeks. Perithecia in the agar plate cultures were all sterile or abortive save two which contained immature ascii without ascospores. The walls of these perithecia were exceptionally brittle, carbonaceous and lacked much of the floccose layers associated with perithecia in nature. These agar cultures were stored in full sunlight and slowly dried, but no decided change in the formation of perithecia was noted.

In one culture where the snowberry was inoculated, perithecia formed on the fifth week of incubation. These perithecia were on a floccose stroma but not buried in it. The stroma averaged 60 microns deep and was located on the surface of the snowberry (PLATE 17, FIGS. A, B, C). Perithecia were most numerous within wrinkles or between folds or those surfaces which did not have free access to the air. Perithecia bearing viable ascospores were collected in snowberries on diseased shrubs in 1928 and in 1929, thus showing that the ascogenous stage may appear late in the autumn while the snowberries are attached to the shrubs. Perithecia were also found on old mummies which had overwintered under leaves. Viable ascospores were expelled in April.

Hand sections of matured perithecia were mounted in green lactophenol (PLATE 17, FIG. D). The following table shows the average measurements in microns of 25 perithecia and ascii, and 100 ascospores, together with measurements reported by two other investigators:

The perithecia generally occurred singly but sometimes two or more were joined at their bases. The wall was carbonaceous and

TABLE

Authority	Substrate	Width of Base	Total Height	Break		Asci	Ascospores
				Width	Length		
The writer.....	Snowberry	164-312 <sup>4</sup>	246-451	50-70	50-110	10-14.4 X 63-72 12 X 72	5.4-7.2 X 14.4-20
		213 <sup>5</sup>	344	57	81	6 X 16	
Spaulding and von Schrenk	Apple agar	—	125-250	—	—	9 X 55-70	3.5-5 X 12-22
Craig .....	—	—	—	—	—	16-64	5-7 X 20-28

<sup>4</sup>Limits of variation.<sup>5</sup>Standards based on 60 per cent or more of the individuals examined.

somewhat brittle. From the table above it is to be noted that they averaged 213 microns wide at the base and 344 microns in height. They had three layers: an exterior consisting of floccose hyphae, a central of thick-walled hyphae, and an interior of hyaline thin-walled hyphae. Most of the perithecia on snowberries possessed distinct beaks which averaged 57 microns wide at the base and 81 microns long or occupied about one fourth of the total height of the perithecium. A few hyphae, "hairs," were sometimes found on the beak. The shapes of perithecia and beaks differed; a globose perithecium with a conical beak; a cylindrical perithecium with a conical and reflexed beak; both perithecium and beak forming an ovoid, and indistinctly defined from each other (PLATE 17, FIGS. A, B, C; PLATE 18, FIGS. B, C).

The asci were oblong, clavate to subclavate, blue at the tip, opened by a pore through the thick-walled tip which may possess a lid. They were mostly attached to the base of the perithecium and sometimes pedicellate (PLATE 18, FIGS. D, E, F). In some perithecia a hyaline material was located between asci and in this material were very minute fibers suggesting paraphyses. However, paraphyses press against the pore of the perithecium and aid the escape of its contents. The ascus wall did not stain with potassium iodide while the contents of the ascus was colored an amber.

The ascospores in the ascus were alantoid, hyaline, uniseriate, non-septate. However, they germinated immediately, even in the ascus, and then some were septate. The asci averaged 12 microns wide and 72 microns long, while the ascospores averaged 6 microns wide and 16 microns long. The ascospores are sometimes forced into the tip of the ascus from which some escaped through a pore (PLATE 18, FIGS. D, E, F). The ascospores are short lived; very much like the whole perithecium in this respect. In cultures, asci and ascospores disappeared in 9 days so that the culture was useless for experimentation. Conidia and ascospores were much alike in appearance and were sometimes found in the same culture. However, the ascospores were allantoid (sausage-shaped) while conidia were cylindrical without the crescent bend; ascospores appeared narrower and generally bore a blue tinge while the conidia were hyaline. On account of

these two spore forms of the fungus being so much alike and so closely associated it was considered unsafe to make a monosporous culture from single ascospores; so asci were isolated and when the germ tubes of the ascospores had started to penetrate the ascus wall, the ascus with germinating ascospores was transferred to a culture tube of potato-dextrose agar. Ten such isolations were made and nine produced the characteristic conidial stage. Five snowberries were inoculated with asci containing germinating ascospores and three showed the characteristic disease together with the conidia of a *Gloeosporium*. Thus the life cycle of the polymorphous fungus was established from conidial to the ascogenous or perfect stage from which it returned to the original conidial or propagative stage.

According to the evidence collected regarding the physiology and morphology of the fungus, it is *Glomerella rufomaculans* (Berk.) Spauld. & Schrenk, or in its conidial form *Gloeosporium rufomaculans* (Berk.) Thüm. (*Gloeosporium fructigenum* Berk.).

#### DISCUSSION

Most of the perithecia cultured on the snowberries had beaks which would seem to be a constant morphological character of the organism on this host. However, Spaulding and von Schrenk (p. 24) stated that the perithecia of *G. rufomaculans* have no beaks. They obtained their perithecia from cultures on apple agar which had been stored at 33° to 38° C. The writer obtained perithecia on snowberries which had been inoculated, incubated at 20° to 23° C., and exposed to natural conditions of light and air. Furthermore, perithecia were collected on infected snowberries in the open where they developed entirely under natural environment. These perithecia which developed under natural conditions could not be distinguished morphologically from those which developed under controlled laboratory conditions.

The fact that perithecia on snowberries were beaked, the walls not entirely carbonaceous but of three layers, of 344 microns in height instead of 250 microns as in the type, with asci and ascospores slightly larger, is no more than to be expected when one considers the afore-mentioned variation of conditions under which the two forms were cultured. However, these morphological

variations are not sufficient to establish a new species since the physiology of the two forms appears the same. So *Glomerella rufomaculans* seems to be the proper Latin binomial for the fungus causing anthracnose of the snowberry.

#### CONTROLS

Two groups of shrubs, each situated on the south side of a building but well separated by a large entrance, were employed for the dusting experiment. The group on the east side of the entrance was dusted while that on the west remained as a check. The group dusted consisted of 270 stems while the check had 300.

The dust chosen was Niagara copper carbonate dust D-6. About one quart of the dust was employed during each application with a hand duster, the plan being to keep the leaves, stems and fruit covered with dust. Care was taken to dust under surfaces of leaves and fruits. The number of applications was reduced to a minimum as the autumn of 1929 was exceptionally dry for Massachusetts.

The first application was made the day following the appearance of the first diseased snowberry, September 2, 1929, and afterwards as follows: September 14, 28, and October 12 when the pink spore masses ceased to form on the snowberries.

Observations for the final results were taken on November 22, 1929, or when the leaves had fallen and the snowberries should be at their height of beauty. The apparently healthy and the diseased snowberries were counted on 200 racemes, which were well distributed among the dusted plants and the checks. The snowberries on each of 100 racemes on the dusted plants averaged: apparently healthy 3.7; diseased 2.7. While the checks showed: apparently healthy 1.6; diseased 2.2 snowberries per raceme. However, the fact is recognized that many of the diseased snowberries had fallen and many racemes on the dusted plants and on the checks were entirely without fruit. Yet, the apparently healthy berries on the dusted shrubs were more than twice the number on the controls, which would seem to be an important fact to recognize. Yet, when so many diseased fruits were internally infected by the fungus advancing from the stem into the fruit, spraying and dusting would only control the dissemin-

nation of the fungus, which probably accounts for the dusting benefits just mentioned.

Sporulating cankers were found on the stems in 1929; so it would seem advisable to spray in the springtime with a dormant spray such as lime-sulphur.

As the parasitic fungus is systemic, it would also seem advisable to remove all canes bearing diseased fruits by pruning them to the ground during the dormant season and burning the rubbish. Then, starting with new canes the following season, apply a dormant spray in the springtime and dust in the fall. However, diseased fruits and leaves should be gathered and burned and the soil around the shrubs cultivated.

As a sanitary precaution, apple trees in the immediate vicinity should be properly sprayed and all limb-cankers pruned or properly attended. If the beauty of snowberries is to be retained, pruning, dusting and sanitation should be practiced.

#### SUMMARY

1. An anthracnose of snowberries was prevalent in northeastern United States during 1928. The disease was widely disseminated and prevalent in the New England States during 1927, 1928, and 1929.
2. The injury to the snowberry plants was severe, as about 60 per cent of the snowberries were infected, causing them to drop prematurely and thereby much of the beauty and value of the shrub lost.
3. The disease was caused by a fungus:
  - A. Ascogenous or perfect stage: *Glomerella rufomaculans* (Berk.) Spauld. & Schrenk.
  - B. Conidial or imperfect stage: *Gloeosporium rufomaculans* (Berk.) Thüm. (*G. fructigenum* Berk.)
4. The infection first appeared during the autumn; conidia formed on acervuli and the fungus was disseminated. Under proper conditions, perithecia formed in snowberries and conidia formed in overwintered diseased snowberries during the following spring. It is believed that hyphae overwintered in the buds, advanced into the pith of the new wood, passed along the hollow stem, then outward to the bark, through the flower stalks and the fruits.

5. The fungus infected and formed viable conidia in each of snowberries, coralberries, apples, pears, quinces, tomatoes, bananas, grapes and peppers. It did not infect cranberries.

6. The following controls are suggested:

1. Observe sanitation by destroying diseased plant parts and cultivate the soil around the shrubs.

2. Prune the diseased twigs and stems at the ground, starting with new wood which should be cared for by applying a dormant spray of lime sulphur.

3. Spray apple trees in the vicinity and attend all cankers.

4. Apply copper carbonated dust, beginning at the first appearance of diseased snowberries in September and continuing to keep the snowberries and leaves covered with the dust until freezing weather or sporulation ceases on the snowberries in the open.

## PART II

### ALTERNARIOSE OF THE SNOWBERRY CAUSED BY ALTERNARIA SOLANI FORMA SYMPHORICARPI

Barrus and Horsfall (1) reported an *Alternaria* in the tissues of the snowberry and that it caused water-soaked lesions. They isolated the fungus from stems of the snowberry plant and inoculated by puncturing the fruit and produced the disease. However, the life history and species of the fungus remained unknown.

Young (11) listed an *Alternaria* on the snowberry and believed it to be a physiological form of *Alternaria tenuis* Nees, or species No. 1, as reported by Saccardo (6). Young inoculated wheat and oat seedlings with conidia of an *Alternaria* which he had isolated from the snowberry, obtained infection and assigned the fungus to his physiological form nine (P. F. 9). He also reported that this organism did not infect cabbage in the greenhouse.

In the autumn of 1928, many diseased snowberries (*Symporicarpos albus* L.) Blake var. *laevigatus* (Fernald) Blake which were covered with dark hyphae and bore conidia of an *Alternaria* were observed at Amherst, Massachusetts. In 1929, the disease reappeared and experimentation was undertaken for the specific purpose of determining:

1. How may the symptoms of alternariose be distinguished from anthracnose?
2. How important is the disease?
3. What Latin binomial should be assigned to the fungus?
4. How may the disease be controlled?

#### DISTRIBUTION AND SYMPTOMS

Alternariose of the snowberry was very widely distributed during the autumns of 1928 and 1929. In 1928 it was located in several New England States and New York State. In 1929 this disease was more prevalent than during the preceding season when it was sparsely distributed on each shrub examined. During both years it was associated with anthracnose and as prevalent. Actual counts of diseased fruits, in October, showed about 25 per cent of the snowberries on the shrubs bore symptoms of alternariose.

Snowberries infected with alternariose changed from a white color to a buckthorn or a dresden-brown, but when old they were a mummy-brown while those lately infected with anthracnose were rufous or red instead of yellow. Furthermore, snowberries infected with alternariose generally became soft and watery and, upon long standing, were covered with grayish or olive-black mycelium while those infected with anthracnose were rufous, finally turning to a dead black, wrinkling and mummifying with the surface often remaining smooth and without mycelium. However, both fungi causing these two diseases were often cultured from the same diseased parts of fruits and twigs of the snowberry plant (PLATE 19, FIG. B, 1-5; FIG. D).

#### ISOLATIONS AND INOCULATIONS

Monosporous cultures were made from conidia of an *Alternaria* which had completely covered several snowberries with its mycelium (PLATE 19, FIG. B, 1-5). Healthy snowberries were inoculated by placing conidia and mycelium from a monosporous culture in punctures, on unpunctured surfaces, on the calyx ends and on broken pedicels. Likewise, other snowberries were inoculated with *Alternaria Brassicae* (Berk.) Sacc., which had been isolated from the leaves of common cabbage (*Brassica oleracea*

var. *capitata* L.) and with *Alternaria Solani* (Ellis & Mart.) Jones & Grout isolated from tomato leaves (*Lycopersicon esculentum* Mill.). Proper checks were prepared and the inoculated snowberries were incubated in Coplin breeding jars lined with damp filter paper and stored at 20° C. One series of these inoculations was made in October, and another in November, 1929.

Two weeks subsequent to the inoculations made in October, all the checks and snowberries inoculated with *A. Brassicae* were healthy. The punctured snowberries inoculated with *Alternaria* isolated from the snowberry and from the tomato were infected with an *Alternaria*, the inoculum from the tomato apparently being more active than that from the snowberry. When the inoculum was placed on the calyx end, only three of the 10 snowberries were infected; none of the snowberries with inoculum placed on the pedicels were infected. Thus the *Alternaria* parasitizing the snowberry and *A. Solani* which parasitized the tomato were the same physiologic form. However, the results of the November inoculations were somewhat confusing as so many of the snowberries apparently healthy were internally infected and this infection was apparent only when the snowberries were brought from the open and placed within a damp chamber stored in a warm room (20° C.). Even then, some of the snowberries which had remained healthy for several weeks suddenly showed infection. In one series, 40 apparently healthy snowberries were divided into four lots of 10 each and each lot treated as follows: Lot 1, check; Lot 2, inoculated with *A. Solani*; Lot 3, inoculated with *A. Brassicae*; Lot 4, inoculated with a monosporous culture of an *Alternaria* from the snowberry. The surfaces of the snowberries were sterilized for 8 minutes in an aqueous solution of bichloride of mercury, 1 : 1000, to which had been added 2 cc. of HCl, washed in distilled water, inoculated and incubated in a sterilized Coplin breeding jar. After two weeks' incubation, each snowberry inoculated with an *Alternaria* from the tomato and from the snowberry was infected with an *Alternaria* which was sporulating. Three of the checks and four inoculated with *A. Brassicae* were healthy. However, snowberries in each series were infected with a *Gloeosporium* and one

or more in each series with an *Alternaria*. The cold weather in November had checked the activity of the pathogen in the open but its activity was resumed when the host parts were transferred to a growing temperature in the laboratory. Also, the *Alternaria* from tomato infected the snowberries, even though they were infected by anthracnose. However, from these inoculations it is to be noted that the *Alternaria* parasitizing the tomato and that parasitizing the snowberry are of the same physiological strain.

Leaves of potted Bonny Best tomato plants were inoculated with monosporous cultures of *Alternaria* isolated from the snowberry and from the tomato. Mycelium and conidia were spread on both upper and under leaf surfaces. Before inoculation, the soil in the pots was soaked with water and the plants atomized with distilled water. The inoculated plants were covered with bell jars lined with damp paper and stored in the greenhouse at a temperature averaging 24° C. Ten days afterward, the tomato leaves had been infected by conidia of *Alternaria* from both snowberry and tomato, while the checks were apparently healthy. Several of these lesions on the tomato plants bore the characteristic targeting and conidia of an *Alternaria*. This experiment was also duplicated by others (college students) with like results. These inoculations showed that the *Alternaria* parasitizing tomato leaves was the same physiologic form as that which parasitized snowberries.

Brown hyphae of an *Alternaria* were located in the palisade and mesophyll layers in the leaves of the snowberry plants and sometimes emerged through the stomata. However, the targeting which generally accompanies alternariose of leaves was not observed on leaves when collected in the open. When leaves of the snowberry plant were inoculated with the monosporous culture of *Alternaria* from the snowberry employing Clinton's method (2), they turned a dark gray color after three days' incubation and then the fungus seemed more active as a saprophyte on dead leaf tissues than as a parasite on the living host.

The *Alternaria* was also cultured from stems of shrubs. In July, 5 twigs which had borne diseased snowberries during the previous autumn were sterilized for 8 minutes in an aqueous

solution of bichloride of mercury (1 : 1000 with 2 cc. of HCl added), washed in distilled water, cross-sectioned through each node and internode of each year's growth and these sections incubated on potato dextrose agar. The results showed that over half of the sections contained hyphae of an *Alternaria*. The fungus was most prevalent in sections removed from the nodes and in the outer bark. Microscopic examination of free-hand sections adjacent to those which were incubated on the agar showed that *Alternaria* hyphae were under the bark in abundance and that they sometimes sporulated on the surfaces of both young and old canes. Furthermore, *Glomerella rufomaculans* was generally present in the cultures and the two fungi seemed very closely associated. No evidence is at hand to determine whether the *Alternaria* followed the path of penetration made by the *Glomerella* or vice versa or whether they were symbiotically associated.

During the winter of 1928-29, an *Alternaria* was cultured from the bark and from the buds of snowberry shrubs. These experiments seem to present evidence that the hyphae of the *Alternaria* were perennial in the bark and bud scales of the snowberry shrub and especially plentiful at the nodes.

#### MORPHOLOGY AND TAXONOMY

Fresh conidia were removed from naturally infected snowberries and measured by employing the proper technique for such work. The measurements in microns follow: with no crosswalls,  $8.5 \times 12$  microns; 1 crosswall,  $12 \times 19$ ; 2,  $13 \times 21$ ; 3,  $14 \times 27$ ; 4,  $12 \times 31$ ; 5,  $15 \times 37$ ; 6,  $22 \times 43$ ; 7,  $14 \times 46$ , and with 8 crosswalls,  $14 \times 51$  microns. Standard;  $14 \times 27$  microns; 3 crosswalls; muriform; 72 per cent (100 measured and the pedicels excluded). Variation,  $8.5-17 \times 12-51$  microns. Forms varying from clavate with 8 crosswalls and muriform to those globose with one cell (TEXT FIGURE 4, NUMBERS 3-11); pedicellate or non-pedicellate; pedicels  $2 \times 2-12$  (standard) or sometimes 80 microns long; conidia catenulate, varying to the *Stemphylium* type (TEXT FIGURE 4, 1, 2, 10, 11). The conidia were slightly constricted at the crosswall when the spores were in chains and attached but decidedly constricted at the crosswalls when germinating

and somewhat constricted after having been submerged in water for some time. The colors of immature conidia varied from a buckthorn to a dresden brown while matured conidia were a mummy brown.

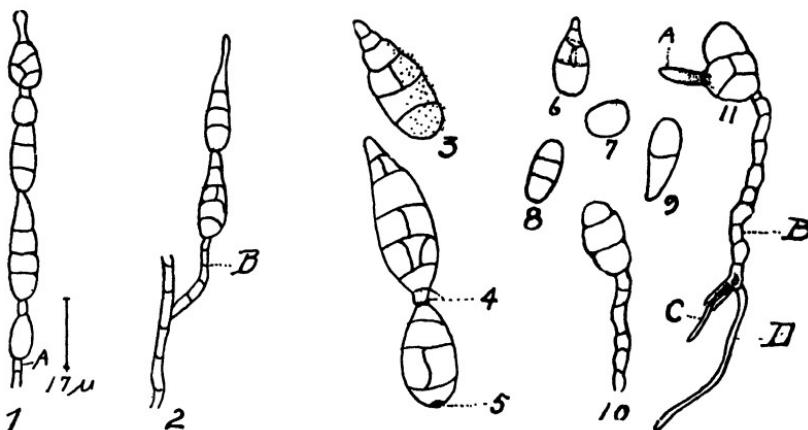


FIG. 4. Tracings from outlined, camera-lucida drawings showing various types of conidia and conidiophores of *Alternaria Solani* which parasitized the snowberry. No. 1-2. Chains of conidia as they formed in a drop slide cultures of carrot decoction which originally contained a single conidium removed from the snowberry. The *Alternaria* type of conidium (catenulate) is evident. No. 1A, and No. 2B, the original or primary conidiophores; No. 3-10. Conidia removed from the snowberry; No. 3. A minutely spinescent conidium; No. 4-5. Large conidia; No. 7-10. Conidia bearing cells varying from 1 to 5 in number; No. 10. The *Stemphylium* type of conidium; No. 11. A, C, D Germtubes of a conidium which had been submerged in water for 20 hours. Cells of the pedicel (B) formed germtubes (C and D). Pedicellate cells did not "germinate" when old spore materials were employed.

Measurements of conidia of *Alternaria* from the snowberry and from the tomato as reported by several investigators:

Investigator	Tomato	Hosts	Snowberry
Young (4) . . . . .	7-20 X 14-60 microns 11-14 X 14-56 11-14 X 40-50	Pedicels included	7-18 X 15-35 microns <i>Alter. tenuis</i> (Young's) 14-15 X 30-36
Saccardo (3) . . . . .	Macrosp. tomato 20-22 X 100-120		
Rands (2) . . . . .	Same as on potato 12-20 X 120-296		
Davis . . . . .	12-20 X 18-160 Pedicels omitted (80 microns)	8.5-17 X 12-51 14 X 27 (standard)	

Considerable variation in the sizes of conidia is to be noted in

the above table. However, mycologists generally concede that spore measurements are of little, if any, aid in determining a species of *Alternaria*. Young (11) reported conidia of an *Alternaria* parasitizing the snowberry as measuring  $7-18 \times 15-35$  microns. He included the pedicels in his measurements. Saccardo (3) gave the conidial measurements of *A. tenuis*, of which Young believed the fungus on snowberry a physiological form, as  $14-15 \times 30-36$  microns. He also described the conidia as muriform, 3-5-septate and constricted at the septa. Young tabulated measurements of *Alternaria* on *Lycopersicon* and a wide variation in sizes is to be noted.

The measurements of conidia from the snowberry as reported by the writer vary from those reported by Young and those reported by others for *A. Solani*. The environmental conditions play such an important part with the sizes of conidia that this variation is to be expected. These measurements of conidia do not vary sufficiently to exclude this *Alternaria* from *A. tenuis*, considering variation in the sizes reported by Young and Saccardo. However, these conidia are shorter, beaks less prominent and shorter than those of *A. Solani* as reported by Rands (4), who believed that *A. Solani* is the same species of *Alternaria* as that which parasitizes the Irish potato (*Solanum tuberosum* L.). The action of *A. Solani* and of the *Alternaria* on the snowberry was physiologically the same but, considering the sizes of the conidia and pedicels, the two fungi were morphologically different. In view of the fact that at the present time there is no accurate method of classifying an *Alternaria* by spore measurements, the physiology of the fungus is the more reliable. However, this fungus is a morphological variation and a physiological form of *A. Solani*. Perhaps many physiologic forms exist but, until a definite scheme of classification is presented to mycologists, all these classifications remain arbitrary. Since no perfect stage of the fungus was observed, a tentative classification is suggested for the organism causing the anthracnose of the snowberry: *Alternaria Solani* (Ellis & Mart.) Jones & Grout forma **Symporiorum** forma nov.

### CONTROLS

On September 2, 1929, snowberry shrubs were dusted with Niagara copper carbonate dust D-6. The plants were afterwards dusted on September 14, 28, and October 12, or when the growing season had passed. The leaves, stems, and fruits were constantly covered with the dust during September and October. In November, when the berries should be at their height of beauty, observations were made and recorded. Only five snowberries with *Alternaria* could be found on the dusted plants while a good number were to be noticed on the undusted checks. As this fungus was so often associated with the *Glomerella rufomaculans* no definite check or counts could be obtained. However, dusting under the conditions observed checked the fungus. Since the fungus resides in the canes, all rubbish from pruning the shrubs should be burned together with the old diseased snowberries and the soil around the base of the shrub thoroughly tilled. As conidia of *Alternaria* overwinter in this latitude and viable mycelium was found in the bark under winter condition, it would seem advisable to employ a dormant, spring spray of lime sulphur.

### SUMMARY

1. The symptoms of alternariose may be distinguished from anthracnose as follows: snowberries infected with alternariose were yellow or brown (buckthorn, dresden- or mummy-brown) color, while those with anthracnose were red or black (rufous to olive or dead black). A soft watery rot generally accompanied alternariose while a dry rot, wrinkling and mummifying of the snowberries accompanied alternariose.
2. Alternariose is important since it may cause 25 per cent of the berries to rot. It also parasitizes the bark of the cane and the bud scales.
3. The Latin binomial of the fungus is *Alternaria Solani* (Ellis & Mart.) Jones & Grout. This classification is principally based on its physiological behavior. However, if both the physiological and morphological characters are to be considered, *Alternaria Solani* (Ellis & Mart.) Jones & Grout f. (forma) *Symporicarpi* is suggested.

4. The disease was controlled by keeping the stems, leaves, and snowberries covered with copper carbonated dust during September and October.

Pruning, sanitation, tillage of the soil around the shrubs and application of a dormant spray (lime sulphur) in the spring are suggested controls.

### PART III

#### A BOTRYTIS ROT OF THE SNOWBERRY CAUSED BY BOTRYTIS VULGARIS

In October 1928, infected snowberries of a yellow-ochre color were observed on all snowberry shrubs inspected. As the cause for this yellowing of the fruit was unknown, ten of these yellow snowberries were placed in a Coplin breeding jar lined with damp filter paper and stored at room temperature. Three days afterwards, most of the snowberries were covered with a pale smoky-gray mycelium. After five days' incubation, conidiophores bearing several clusters of hyaline conidia of the *Botrytis* type appeared on the surfaces of several snowberries (PLATE 18, FIG. A, 3; PLATE 19, FIG. B, 11, 12, 13).

#### ISOLATION AND INOCULATION

The fungus was isolated by transferring conidia on conidiophores to potato-dextrose agar, employing the technique common to such procedure. Pure, sporulating cultures were readily obtained.

For inoculating purposes, forty snowberries were removed from a plant bearing a low percentage of infected fruit (50 per cent). The surfaces of these snowberries were sterilized for 8 minutes in an aqueous solution of bichloride of mercury, 1 : 1000, to which 2 cc. HCl had been added. The inoculum consisted of conidia and mycelium from pure cultures obtained by methods previously described. The snowberries were inoculated in four series of 10 each; series A, punctured and the inoculum inserted; series B, inoculum spread on the surface (sides); series C, inoculum spread on the calyx end; series D, checks consisting of five punctured and five unpunctured snowberries. The inoculated snowberries were then incubated in a Coplin breeding jar, lined with damp filter paper and stored at 18° C. (11-16-29).

Five days after the inoculation, each inoculated snowberry was entirely discolored by the *Botrytis*, having turned from its normal snow-white to an antimony yellow or yellow ochre. Pale smoky-gray floccose mycelium covered the surface of three snowberries and, on the next day, citron-drab to clove-brown conidiophores bearing aggregates of hyaline conidia of a *Botrytis* were observed.

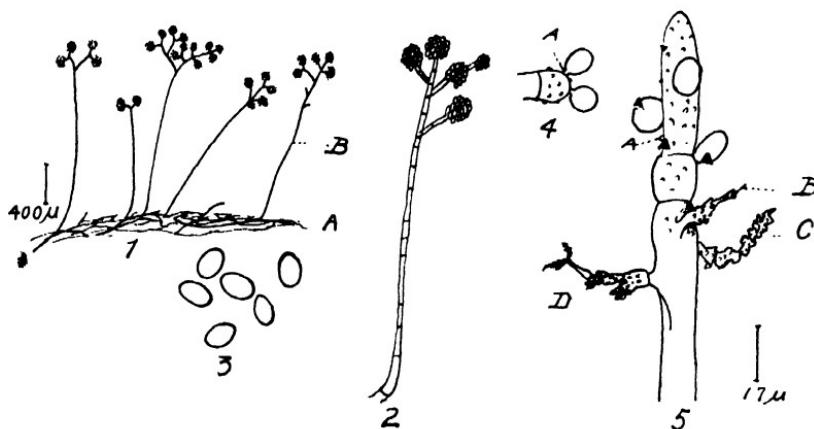


FIG. 5. Tracings from outlined, camera-lucida drawings of conidia and conidiophores of *Botrytis vulgaris* Fries, removed from snowberries. No. 1. Grape-like clusters of conidia on various types of branching conidiophores as observed on the snowberry; No. 2. Somewhat enlarged and diagrammatic representation or a conidiophore showing the cross-walls and enlarged base; No. 3. Various shapes of the conidia, nearly circular, elliptical and ovate in outline; No. 4. The end of a living conidiophore showing conidia attached by small projections representing sterigmata; No. 5. A conidiophore from which most of the viable conidia have been removed. The cells of the branches *B*, *C*, and *D* have collapsed after the conidia matured. The surface (*A*) is roughened by projections showing where conidia were attached.

Leaves of the snowberry plant were inoculated and, after three days, the fungus had turned them a dark-brown color and was sporulating abundantly on their surfaces. The fungus was also collected on leaves in the open. Diseased leaves were removed from the snowberry plant, stored in a damp chamber and, on several occasions, some showed symptoms of a *Botrytis* disease. Furthermore, the fungus sporulated abundantly on some leaf surfaces.

## PATHOGEN

The conidiophores were mostly erect, averaged 2 mm. in length; 12 microns in diameter; 5-septate (3-15 septa); mostly dichotomously branched, 1 to 4 times (TEXT FIGURE 5, NUMBER 1); bore 1 to 12 clusters of conidia on short, obtuse or rounded, hyaline branches which soon shriveled (No. 5). Conidia were somewhat persistent, mostly on secondary conidiophores (branches); hyaline when young but tinged with fuscus when matured; globose to ovate or elliptical (No. 3); measurements in microns of 100 fresh conidia; limits, 6.5-14  $\times$  8.5-19; standard, 9  $\times$  14. Sterigmata, when present, averaging 1  $\times$  3 microns (No. 4). No microconidia were observed. Sclerotia, in cultures, plentiful, nearly black, globose or cylindrical to flat, somewhat irregular in outline, 1 to 7 mm. When cultured, produced mycelium and conidiophores but no apothecia.

The above description compares favorably (5) with that of *Botrytis vulgaris* Fries, as recorded by Saccardo; more especially of the *Botrytis furcata* Fries type.

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## EXPLANATION OF PLATES

## PLATE 15

Photographs showing anthracnose and photomicrographs illustrating the epidermal cells of the snowberry. Materials used in Figs. C, E, and F were from the same snowberry and treated at the same time. Fig. A. Clusters from two different shrubs collected in November: 1, 2, Diseased snowberries with acervuli on the surface; 3, 4, Mummies or old, shriveled, diseased snowberries. Fig. B. Different stages in the development of anthracnose: 1, 2, 3, Initial symptoms of the disease, areas which have turned a rufous color; 4, 5, Diseased areas turned rufous color and mycelium on the surface; 6, 7, After 5 to 7 days, pinkish conidial masses indicated the position of acervuli and were characteristic of the disease; 8, A snowberry six days after inoculation by puncturing the epidermis at the side of the berry and inserting mycelium. Fig. C. Epidermal cells of a dried, healthy snowberry mounted in lactophenol for 8 weeks: 1, 2, 3, 5, Suberized protoplasmic membranes; 4, Cell walls. Fig. D. Epidermal cells removed near the calyx cup of a young snowberry; mounted in lactophenol for 48 hours: 1, A stoma with chlorophyll in the guard cells; 2, Suberin on the upper and side walls. Fig. E. Cells boiled in a 1 per cent potash solution for 15 minutes removed after 15 hours and mounted in lactophenol: 1, Cell wall; 2, 3, 4, The cells now contain globules of suberin material somewhat oily in appearance. Fig. F. Suberin granules after the epidermal cell walls were removed by boiling in a 10 per cent solution of sulfuric acid and mounted in lactophenol: 1, Suberin granules at the side of the cells; particles are also to be seen in the center of the cell lumen; 2, Suberin particles on the side and near the floor of the cell.

## PLATE 16

Photographs of snowberries showing symptoms of anthracnose; also, photomicrographs of acervuli and conidia of *Gloeosporium rufomaculans*. Fig. A. These snowberries had been collected and stored in a damp chamber overnight before photographed: 1, 2, 4, 14, 16, Early stages of infection; 6, 12, 18, 19, Mummies from which perithecia form; 5, 7, 8, and others show the fruiting stage; spore masses on the surface; 3, 5, 9, 17, Early stages in the wrinkling of infected snowberries. Fig. B. Photomicrographs of an exceptionally large young acervulus which had been triple stained and permanently mounted: 1 and 2, Cutin which has been raised by the hyphae; 3, Conidium on its conidiophore in an acervulus; 4, 7, 10, Hyphae of the fungus; 5, Wall of an epidermal cell; 6, Wall of a mesocarp cell. Fig. C. An acervulus one week old. Prepared as in Fig. B. (Part of the background in the negative was opaqued): 1, Deliquescent hyphae; 2, Conidia in gelatinous material; 3, Subcutaneous hyphae. Fig. D. Photomicrograph showing different sizes, shapes and contents of conidia.

## PLATE 17

Photomicrographs of perithecia which had been removed from snowberries and mounted in green lactophenol. Fig. A. Perithecia separated by dissection: 1, 2, 3, Beaks of perithecia; 4, 5, 6, "Hairs." Fig. B. Perithecia on the stroma: 1, The floccose stroma with perithecia in place; 2, Ascospores

escaping through the pore of a perithecium. Fig. C. Hand sections of perithecia near the outer walls: 1, Loose hyphae on the outer walls; 2, Floccose hyphae lining the inner walls; 3, Thick-walled hyphae composing the middle wall; 4, Floccose stroma. Fig. D. Hand sections of a perithecium showing asci in place.

#### PLATE 18

Photographs showing infected snowberries bearing fruiting organs of several fungi. Also, the structure of an ascus and ascospores of *Glomerella rufomaculans*. Fig. A. Nos. 4, 8, Snowberries infected by *Penicillium* sp.; 3, 7, Infected with *Botrytis vulgaris*; 1, 2, 5, 6, Infected by *Gloeosporium rufomaculans*. Fig. B. Perithecia on snowberries: 1-6, Inoculated with a pure culture and incubated in a moist chamber 21 days at 20° C.; 7-9, Diseased snowberries collected in the field and incubated as in Nos. 1-6 above. Fig. C. An enlargement of Nos. 2 and 5 in Fig. B: 1, Perithecia with beaks protruding; 2, Mycelium with conidia; 3, Immature perithecia. Fig. D. The contents of an ascus mounted in green lactophenol: 1-5, Ascii containing ascospores; 6-7, Two ascospores. Fig. E. Photomicrograph of an ascus showing in F which has been traced with ink: 1, Foot of the ascus; 2, One of the allantoid ascospores which has germinated within the ascus. Fig. F. Photomicrograph of the ascus traced in Fig. E.

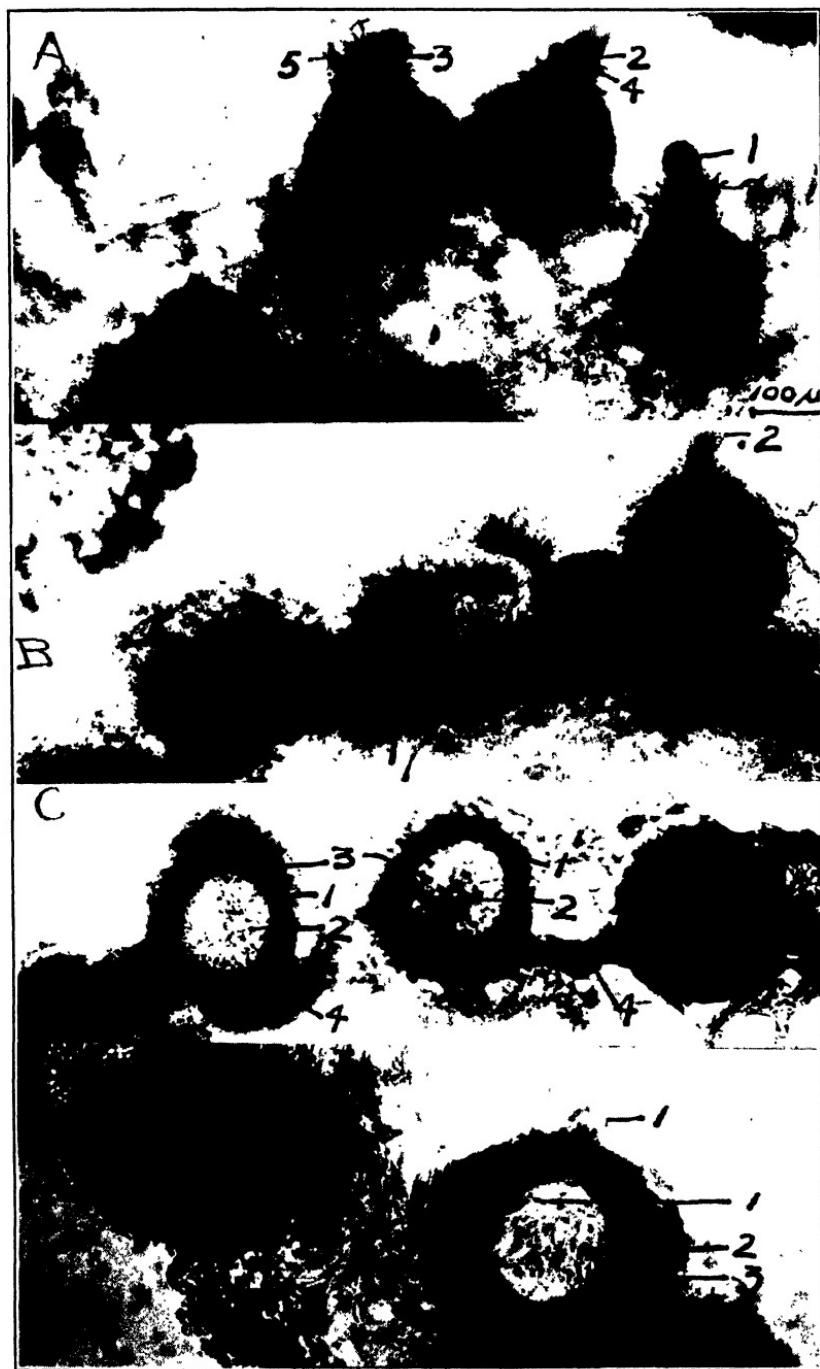
#### PLATE 19

Photomicrographs of the external layers in the fruit and bark of snowberry plants together with the symptoms when the fruit is infected by *Gloeosporium*, *Alternaria* and *Botrytis*. The materials were hand-sectioned and mounted in green lactophenol for one month before photographing. Fig. A. Mycelium of *Glomerella rufomaculans* within the epidermal cells of an infected snowberry: 1, 2, 3, Hyphae passing through pits in the cell walls; 4, 5, 6, 7, Hyphae engulfing nuclei. Fig. B. Two twigs bearing snowberries infected with *Gloeosporium*, *Alternaria*, and *Botrytis* (10-21-29): 1-5, Snowberries infected with *Alternaria Solani* forma *Symporicarpi*; 7, 8, 9, Snowberries infected with *Gloeosporium rufomaculans*; 10, 11, 12, Snowberries infected with *Botrytis vulgaris*; 13, Leaf of a snowberry plant infected with *B. vulgaris*. Fig. C. Perithecia and epidermis removed from a snowberry which had been collected from a twig during October. These materials had been mounted for three weeks in green lactophenol, and a cover glass pressed on them so as to cause the contents to emerge: 1, 2, 3, Perithecia with pores; 4, 5, 7, 8, Ascospores; 6, Epidermal cells of the snowberry. Fig. D. Outer bark from a diseased twig of a snowberry plant infected with *Alternaria Solani*: 1, Mycelium on the surface; 2, 3, Cells of the mycelium forming rounded fuscous bodies analogous to chlamydospores; 4, 5, Conidiophores; 6-9, Mycelium within stem tissues.



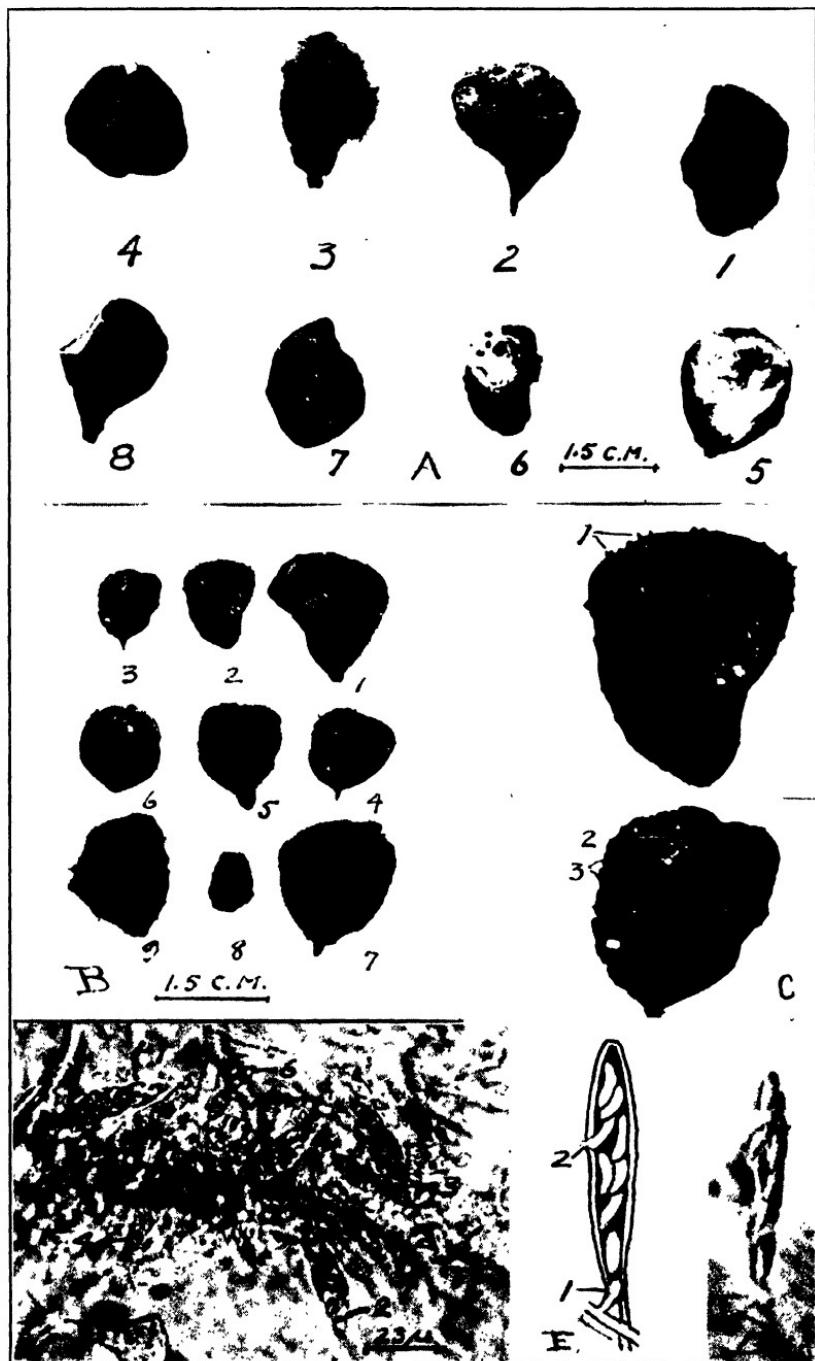
ANTHRACNOSIS OF THE SNOWBERRY





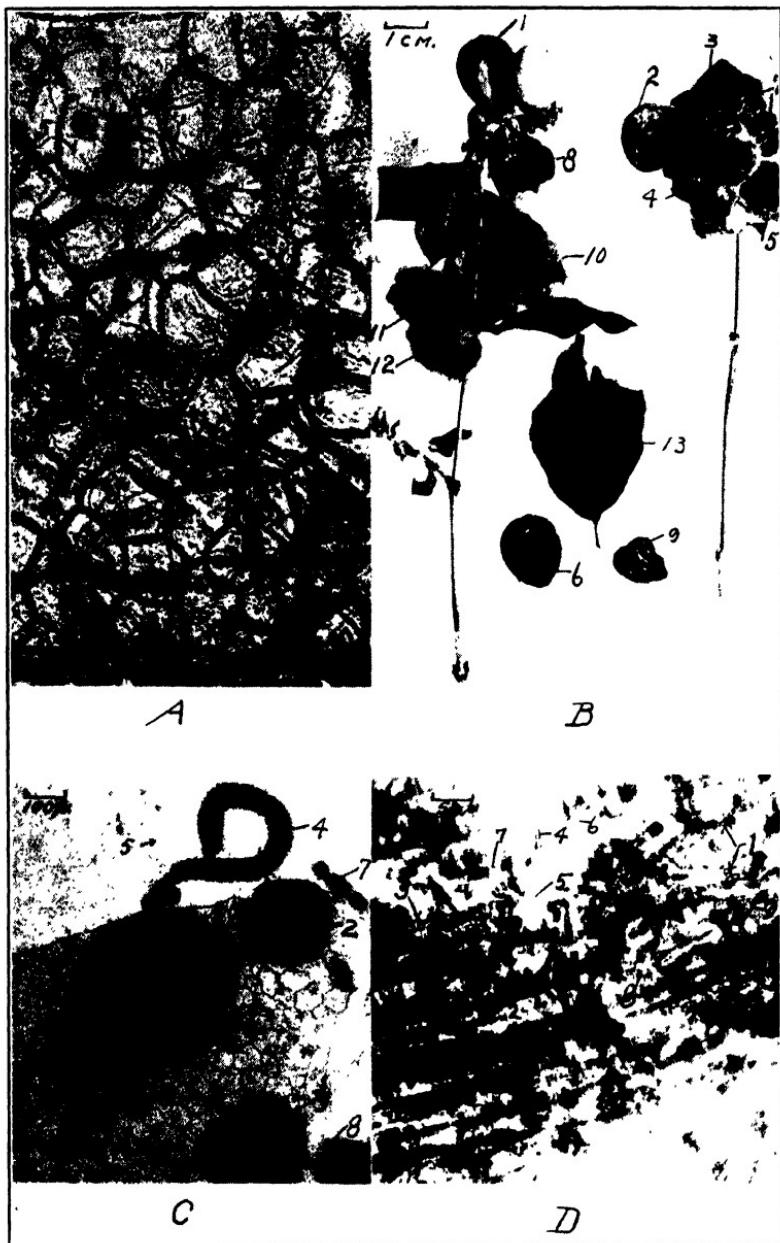
ANTHRACNOSE OF THE SNOWBERRY





ANTHRACNOSIS OF THE SNOWBERRY





ANTHRACNOSIS OF THE SNOWBERRY



## OBSERVATIONS ON PYTHIUM DICTYOSPORUM

FREDERICK K. SPARROW, JR.

(WITH PLATE 20 AND 1 TEXT FIGURE)

Although other members of the genus *Pythium* have in many instances been studied at length, *P. dictyosporum* of Raciborski has received but brief attention since in 1891 it was first reported by him parasitic in *Spirogyra insignis* (Hass.) Kütz., at Cracow, Poland (6). The following year in a paper<sup>1</sup> in Polish he described the fungus more fully and illustrated various stages in the life history of the organism (7), which he then stated was found parasitic in *Spirogyra nitida* (Dillw.) Link. In 1895 under the name *Nematosporangium dictyosporum* (Racib.) Schröter, de Wildeman reported the fungus from France in an unnamed species of *Spirogyra* (10). So far as the writer has been able to ascertain, these are the only published records of the occurrence of the fungus. In October, 1926, however, in a laboratory culture of *Spirogyra crassa* Kütz., collected at Belmont, Mass., the present writer found within this alga the golden reticulate oöspores (FIG. 1, A, B) and, later, the sporangia of this fungus. Additional knowledge of the life history and morphology of *P. dictyosporum*, resulting from a study of the fungus as found in this algal culture, as well as under conditions of pure culture, is presented in the following paper.

### METHODS

By micro-manipulation with fine platinum needles, oöspores with attendant mycelium as well as oöspores alone were separated from the infected algal filaments, that had been washed in sterile water, and were planted in petri dishes of nutrient agar. Colonies of the fungus were readily secured in this manner and by repeated transference bacteria-free cultures were obtained. Non-sexual reproduction was induced in pure culture by thoroughly

<sup>1</sup> The writer wishes to express his thanks to Professor Leo Wiener of Harvard University for his kindness in translating portions of Raciborski's paper.

washing mats of mycelium grown in pea or bean broth and transferring them to sterile distilled water. At room temperature ( $21^{\circ}$  C.), mycelia usually produced zoospores in 10–15 hours after this treatment.

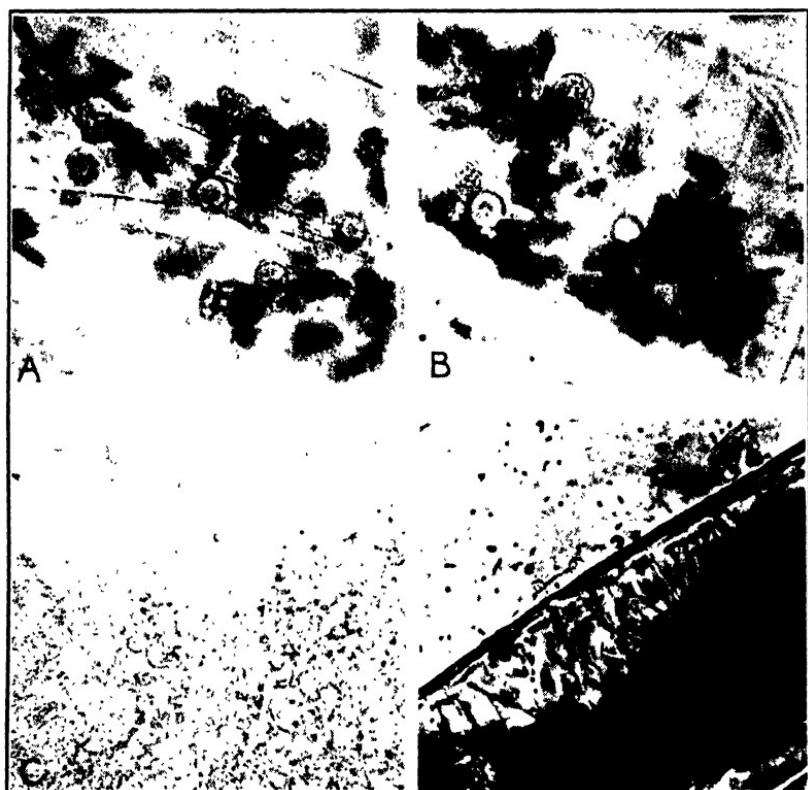


FIG. 1. *A*, General habit of the fungus within the *Spirogyra* cell. Several oospores are shown.  $\times 550$ ; *B*, Similar to the preceding, showing more clearly the character of the oospore walls.  $\times 550$ ; *C*, Mycelium of the fungus which has been grown in pea broth, ten days after its transference to distilled water. The distorted condition of the hyphae may be appreciated by comparing them with the typical, slender ones near the top of the photograph.  $\times 500$ ; *D*, A cell of *Spirogyra* which has been penetrated by the germ tubes of a number of cystospores, the empty shells of which may be seen adhering to the outer wall of the alga.  $\times 550$ . Reduced one third.

#### MORPHOLOGY AND DEVELOPMENT

Within the *Spirogyra*, the fungus formed a non-septate mycelium which perforated the end walls and occasionally sent out

branches even through the lateral walls of the host. The hyphae were isodiametric, hyaline, and finely granular, averaging  $1.8\ \mu$  and seldom being over  $2\ \mu$  in diameter. In pure culture on various types of media, both liquid and solid, the branching was more profuse. A slight increase in diameter was also noted, the filaments generally attaining  $2.5$ – $3.0\ \mu$ , save on 1 per cent proteose-peptone agar, where they frequently reached  $5.4\ \mu$ .

Several specialized vegetative structures not reported by either Raciborski or de Wildeman were observed under certain conditions in this species. For example, the mycelium, when grown on 5 per cent maize oil agar and in such solutions of proteins as 1 per cent peptone or 1 per cent proteose-peptone, was characterized after several weeks by the formation of sub-spherical terminal or intercalary hyphal swellings at frequent intervals along its filaments (PLATE 20, *D*, *E*, *F*). These bodies, which were not cut off by septa from the hyphae, possessed a densely granular content and numerous refractive granules which turned orange when stained with Sudan III.

Moreover, appressoria were also found to develop. These structures did not occur in *Spirogyra*, nor had they been seen by Raciborski and de Wildeman. However, during the course of a series of inoculation experiments, to be described in a later paper, they were found to be formed when the mycelium came into contact with the walls of *Tolypothrix* sp. (?) and *Rhizoclonium hieroglyphicum* (Ag.) Kütz. (PLATE 20, *B*, *C*). The tip of the hypha, coming into contact with a cell wall of the alga, adheres to it by means of a refractive, possibly mucilaginous, substance, slowly becomes distended, and forms a somewhat pyriform structure. This appressorium is securely anchored to the algal cell wall by a disk of cementing substance which is often easily visible. Penetration of the cell wall is accomplished by means of a slender refractive tube produced at the tip of the appressorium. After penetration, there is formed at the distal end of this tube a hypha of ordinary diameter, which by further growth and branching establishes itself within the host cell (PLATE 20, *B*).

One further point regarding the vegetative thallus of this fungus seems worthy of mention. Bits of mycelial mats, grown in some liquid medium, when placed without washing in capsule

dishes and covered with sterile distilled water, after 7–10 days, showed greatly swollen and distorted hyphae (FIG. 1, C). These were formed by the slow accumulation of protoplasm within certain hyphal regions and by the consequent distention of these parts. They did not seem to perform any particular function, although they were observed over a period of ten days. In certain closely allied species, under conditions favorable for non-sexual reproduction, similar appearing bodies are formed as a sub-basal part of the filamentous sporangium. It is of interest to note the absence of such reproductive activity in this instance.

#### NON-SEXUAL REPRODUCTION

The sporangia of *P. dictyosporum* are not differentiated from the vegetative hyphae, but are merely slender branches of the extramatrical mycelium which, with little or no constriction in diameter, grow out through the lateral walls of the host (PLATE 20, A).

The successive changes which culminate in the production of the zoospores in this species do not differ materially from those to be described in some detail for a closely related species in a forthcoming paper. They conform, in the main, to Raciborski's meagre account, as well as to the more lengthy descriptions of Ward (8) and Butler (1) for several congeneric forms, and hence, save for one detail, will be treated very briefly here.

Little information is available regarding the rather critical changes occurring in what has been termed the "sub-apical" region of the sporangium, immediately preceding the formation of the vesicle. This has possibly been due either to the rapidity with which these stages occur, or to the fact that attention has been fixed on the more obvious enlargement of the brightly glistening apical material.

The appearance of the tip of the sporangium a few seconds before the emergence of its protoplasm is shown in Plate 20, G. It may be seen (in optical section) that the apex is divided into three definite regions: an upper, very refractive, crescent-shaped one; immediately below this, a slightly less hyaline zone; beneath this, a pale, slightly refractive, very finely granular region. The distal end of the latter zone is crateriform, and bears at its apex

a highly refractive bit of material which suggests a pore (PLATE 20, *G*). The protoplasm becomes more densely granular a short distance below the tip. If conditions are favorable for the process, there ensue, in rapid succession, the stages seen in Plate 20, *H*, *I*, *J*. In Plate 20, *II*, the refractive dome has lost its double contour, and has commenced to enlarge, and the expansion of the finely granular, crateriform protoplasm distally has been initiated. No change in the position of the lower, more densely granular material has occurred. This stage lasts only an instant and a third stage immediately ensues (PLATE 20, *I*). Here the hyaline cap has continued to expand, the cone of pale protoplasm has become even more distended, and its refractive "pore" has apparently burst or deliquesced, resulting in the liberation into the vesicle of a very finely granular material. The lower, more granular protoplasm has now started to move toward the tip of the sporangium where it will be discharged into the enlarging vesicle (PLATE 20, *J*). The flow of protoplasm thus initiated continues at a fairly even rate until the contents of the concomitant hyphae are exhausted. Cleavage of the homogeneous protoplasmic mass is then initiated, and the subsequent maturation processes result in the production of a variable number of zoospores in the vesicle (PLATE 20, *L*). When maturing under somewhat foul conditions, the vesicular protoplasm may become quite vacuolate (PLATE 20, *K*).

Thus it may be seen that the finely granular, somewhat hyaline protoplasm with its crateriform apex appears to play an intimate part in the initiation of the ejection of the protoplasm from the tip of the sporangium. That it is of a somewhat different material from the underlying, coarse substance filling the rest of the sporangium is apparent not only from its distinctive physical properties, such as refractivity and expansive power, but from its greater affinity for such stains as muchaematein, ruthenium red, and Bismark brown.

Raciborski (7) indicated that there were four zoospores produced by each sporangium of this species, while de Wildeman (10) gave the number as four, followed by a question mark. In the American material, four swarmers were sometimes observed in a single vesicle, but they were by no means restricted

to this number, more than forty being counted in some instances (PLATE 20, *L*). As the number of zoospores produced in a single vesicle depends upon the amount of protoplasm contained between the delimiting hyphal septa, it appears that Raciborski's fungus differed from the writer's and from all other described species of *Pythium* in the regular intervals at which these walls must have occurred.

The zoospore of *Pythium dictyosporum* is of the laterally biciliate type. Its body, which is  $9.0\ \mu$  long by  $5.4\ \mu$  wide, is not quite so rotund as that described by Weston (9) for the zoospore of *Thraustotheca*, but otherwise the two are similar in general appearance and motility (PLATE 20, *M*). The course taken by the actively moving swarmer has invariably been described by investigators working on similar organisms as spiral, whereas the path actually produced is more accurately described as a helicoid one. After a period of motility, the length of which depends upon the conditions of the surrounding medium, the zoospore comes to rest, its cilia are absorbed (PLATE 20, *N*), and a promycelium is produced (PLATE 20, *O*). Repeated swarming was observed by Raciborski but not by the writer.

The actual penetration of the algal filaments is accomplished in most cases by the zoospore. This body, upon coming into contact with the wall of the *Spirogyra*, becomes quiescent, loses its cilia, and assumes a spherical shape. The body thus formed has been termed by Weston (9), in the case of *Thraustotheca*, a "cystospore," a name which is also convenient to use in the fungus herein described. These bodies, in *Pythium dictyosporum*, are from  $8.5$ – $10\ \mu$  in diameter. From the surface of the spore in contact with the wall of the *Spirogyra*, a fine, hyaline, cylindrical tube is produced, which, as it elongates, penetrates the cell wall of the host. The encysted zoospore is apparently cemented to the wall of the alga before the development of the penetration tube, for zoospores at this stage cannot be separated from the *Spirogyra* filament when the latter is vigorously shaken in water.

After penetration, the content of the zoospore passes through the tube at an imperceptible rate and emerges, surrounded by a wall, into the interior of the *Spirogyra*, the empty cystospore

being left adherent to the outer wall (FIG. 1, *D*). Once inside the host cell, the advancing germ tube assumes the diameter of an ordinary hypha and, without branching, grows forward, apparently seeking the food afforded by the chloroplast. Further growth is characterized by ramification of the hyphae throughout the algal cell and penetration of the end walls of the host, with subsequent infection of the whole filament after four or five days.

#### SEXUAL REPRODUCTION

The sexual reproduction of *P. dictyosporum*, because of the unique structure of its oöspore, is of unusual interest both from the phylogenetic and ontogenetic points of view. The occurrence here, in one of the more "primitive" species of the genus, of an oöspore, the structure of which approaches the complexity attained by higher members of the Peronosporales, has been unrecognized by those who attach phylogenetic importance to this organ.

Raciborski's account of the sexual reproduction is, on the whole, quite complete, although certain structural details of the oöspore, as well as measurements of the sex organs, seem to have been omitted. The formation of the oögonia and antheridia presents no features of interest, being similar to that found in other species of *Pythium*. The antheridium is of the "crook-neck," clavate type, being, when fully mature, about  $20\ \mu$  long by  $8\ \mu$  wide (PLATE 20, *P*). Raciborski states that there may be one or two of these, either androgynous or of diclinous origin, attached to each oögonium. In the American material but a single antheridium, of diclinous origin, was found on each oögonium. The separation of the antheridial protoplasm into a hyaline peripheral layer and a central coarsely granular portion, the formation of a narrow fertilization tube through which a part of the inner "gonoplasm" is discharged into the oögonium (PLATE 20, *P*), and the subsequent maturation of the egg thus fertilized, closely follow Raciborski's description. Only a small portion of the antheridial content seems necessary for fertilization, as no empty antheridia were observed adhering to maturing oöspores, nor were any figured by Raciborski. According to the latter, the process of fertilization required about two minutes.

The oögonium when fully formed is about  $21.5 \mu$  in diameter and is ordinarily terminal in its position. Coincident with the formation of the fertilization tube by the antheridium, the oögonial content contracts somewhat and there is differentiated an outer, narrow layer of pale periplasm and an inner, more dense gonoplasm (PLATE 20, *P*). After fertilization, a series of regularly disposed vacuoles appears in the now coarser periplasm (PLATE 20, *Q*). These enlarge and become more angular (PLATE 20, *R*), indicating the initiation of coalescence of the intervacuolar material. Owing to its being mounted in glycerine, the whole content of the maturing oöspore shown in Plate 2, fig. *R*, has slightly contracted away from a thin wall which probably soon after fertilization has been formed around the periplasm, but which, because of its tenuity, escaped detection. The intervacuolar material continues to coalesce (PLATE 20, *S*), and, with the exception of its midregions, falls away from the periplasmic wall (PLATE 20, *T*), delimiting the reticulations characteristic of the mature oöspore. Coincident with the aforementioned changes, a narrow wall is laid down around the gonoplasm which now is greatly condensed (PLATE 20, *T*).

The mature oöspore lies loosely within the old oögonial wall. The thick integument formed from the intervacuolar material of the periplasm is now a golden color and is raised in a series of reticulations that at their junctures form acuminations, the apices of which are generally in contact with the persistent outer periplasmic wall (PLATE 20, *U*). The regions formerly occupied by vacuoles now contain a homogeneous substance of a very low refractive power, probably derived from the contents of these vacuoles (PLATE 20, *U*). When oöspores are dried on slides, this material, together with the periplasmic wall, collapses upon the reticulations, but resumes its former position when water is added. This suggests that the substance is of a gel-like nature, which affords protection for the spore against excessive drought. Both periplasmic wall and gelatinous substance remain ensconced around the oöspore long after the oögonial and host walls have disintegrated. While several of Raciborski's figures plainly show this enveloping substance and its outer wall, there seems to be no reference to them in the text. Due to

de Wildeman's extremely poor figure of the oöspore, it is impossible to say whether or not they were present in his fungus. The content of the oöspore, surrounded by a moderately thick endospore wall, is similar to other species of *Pythium*, possessing the large central oil globule and the laterally placed, lenticular nucleus (PLATE 20, T, V).

Occasionally, oöspores were observed in which the fertilization tube had persisted after the antheridium and even the host cell had disintegrated. In one case, shown in Plate 20, fig. V, this tube appeared to be continuous with the reticulate wall of the oöspore.

So far as could be ascertained from Raciborski's paper, no measurements of the oöspore were given. In the form found by de Wildeman they were stated as being 14  $\mu$  in diameter. In the present material, they varied from 9–23.5  $\mu$ , the majority measuring 17.5  $\mu$ . Both extremes in size were often found in the same algal cell.

Germination of the oöspores was not observed by the writer or by de Wildeman, although Raciborski obtained it in a few cases after the spores had remained over four months (January to May) in water. The process took place very rapidly and involved the disappearance of the oil globule and the production of a filament which pierced the center of one of the polygonal areas, developed into a sporangium, and formed four zoöspores in the usual manner at its tip.

Inasmuch as the detailed description of *Pythium dictyosporum* Racib. is written in Polish, and hence generally unavailable, and as certain points brought out in this present study show a slight emendation of this species is necessary, it has been thought justifiable to include here a technical description of this form.

#### PYTHIUM DICTYOSPORUM<sup>2</sup> Raciborski.

Bull. Acad. Int. Sci. Cracovie 1891: 283–287.

Kraków Nakt. Akad. Umiejet 24: 1–9, 1 pl. 1892.

*Nematosporangium dictyosporum* (Racib.) Schröter, de Wildeman, Notes Mycologiques VI, Ann. Soc. Belge Microsc. 19: 210–215, pl. 7, figs. 4–14. 1895.

<sup>2</sup> It should be noted that the specific name of this fungus is *dictyosporum* and not *dictyospermum*, as A. Fischer erroneously termed it and as has been perpetuated by practically all later writers.

Mycelium intra- and extramatrical, the latter forming, upon occasion, small, pyriform appressoria; composed of hyphae 1.8–2.0  $\mu$  in diameter, isodiametric, forming in certain media (generally protein) irregular swellings. Zoösporangia undifferentiated from the vegetative hyphae; zoöspores few to many (4 to 40) formed in a vesicle; of the laterally biciliate type, 9.0  $\mu$  long by 5.4  $\mu$  at their greatest width, coming to rest and rounding off into a cystospore, averaging around 10  $\mu$  in diameter; germinating by one or two germ tubes. Oögonia (in *Spirogyra crassa*) 12.6–28.8  $\mu$  (average 21.6  $\mu$ ) in diameter. Antheridium of the crookneck type, 20  $\mu$  long by 8.0  $\mu$  at its greatest width, cut off by a single basal septum, its abruptly tapering apex making narrow contact with the oögonium; forming a fertilization tube of varying length, approximately 2.0  $\mu$  in diameter; one (rarely two) to an oögonium, borne terminally on a lateral branch of a hypha, usually distinct from the oögonial hypha. Oöspores (in *Spirogyra crassa*) one to an oögonium, not filling the oögonium, containing a single oil globule of variable size, surrounded by a smooth endospore wall; possessing a golden exospore wall which is raised to form reticulations, the junctures of which are elevated to form acuminate protuberances, adnate to a slender, persistent, periplasmic wall which encloses a gel-like substance of low refractive power in which the whole oöspore is imbedded; germinating after a period of rest (four months in water) by means of a single hypha which pierces the center of one of the polygonal areas bordered by the reticulations of the exospore, grows to a variable length, and becomes converted into a sporangium, and produces at its tip four zoöspores.

Parasitic in *Spirogyra nitida*, Cracow, Poland.

Parasitic in *Spirogyra* sp. (?), France, de Wildeman.

Parasitic in *Spirogyra crassa*, Belmont, Mass., the writer. (On artificial inoculation found capable of attacking also *Tolyphothrix* sp. (?), *Rhizoclonium hieroglyphicum* and *Cucumis sativus*, as will be described in a later paper.)

#### DISCUSSION

Several points of interest are presented by this study of *Pythium dictyosporum*.

Thus far, all efforts to obtain sexual reproduction in this species on artificial media or in *Spirogyra* inoculated with zoöspores and mycelium from pure cultures have been unsuccessful. This is particularly unfortunate, as a cytological

study of this fungus would be of great interest, especially when compared with such forms as *Albugo* and *Peronospora*. However, work on this point is being continued. The methods used by Klebs (5) and Kauffman (3) to induce sexual reproduction in members of the Saprolegniaceae and those employed by Johann (2) in the case of *Pythium arrhenomanes* Drechsler were tried, but yielded no results. As oöspores, with no attendant mycelium, were used in obtaining some of the original cultures of the fungus, it is not probable that a single strain of a heterothallic form has been kept in cultivation.

There are, in the oöspore of this species, three well defined walls: an innermost one, surrounding the living contents; an outer, thicker layer, raised in a reticulate manner; and an outermost thin, persistent one surrounding the whole. Until detailed cytological evidence is forthcoming on their origin and development, they may be regarded (passing from the innermost to the periphery) as (a) the endospore wall of one layer, probably derived entirely from gonoplasm, (b) the reticulate exospore wall, (c) the outer, smooth, narrow periplasmic wall. The last two are unquestionably derived from periplasm.

King (4), defining the position among the Phycomycetes of *Araiopora*, a member of the Leptomitaceae, called attention to the oögonial origin of the fertilization tube of that fungus. He further pointed out that in *Albugo*, a member of the Peronosporaceae, this structure is formed not only by the antheridium, but, in some instances, partially by the oöplasm. The same writer also emphasized the persistence of the fertilization tube in *Albugo*. Taking *Araiopora* and *Albugo* as end points in a phylogenetic series, King suggested that *Pythium*, with its poorly developed evanescent tubes, "tends in a measure to bridge over the wide gap between them." It has been shown in *Pythium dictyosporum* that, in many instances, the fertilization tube is quite persistent and at times appears to be reinforced by oöplasmic material.

Without entering into a discussion as to the relative significance from a phylogenetic standpoint of the sexual and non-sexual structures of *Pythium*, the writer merely wishes to emphasize the fact that, in *Aphragmium*, the sub-genus containing the

supposedly more primitive species of the genus, there is known at least one form, *P. dictyosporum*, which possesses an oöspore comparable to those of certain of the higher Peronosporaceae.

#### SUMMARY

The present paper describes the finding for the first time in the United States of *Pythium dictyosporum*, a seemingly rare and little known phycomycetous parasite of *Spirogyra crassa* Kütz., the material of which was collected October, 1926, in a small pond near Belmont, Mass.

Details of the less known phases of the morphology and development of the fungus are given, especially with respect to certain critical stages in the formation of the vesicle, the method of penetration of the zoospore, and the structure of the mature oöspore.

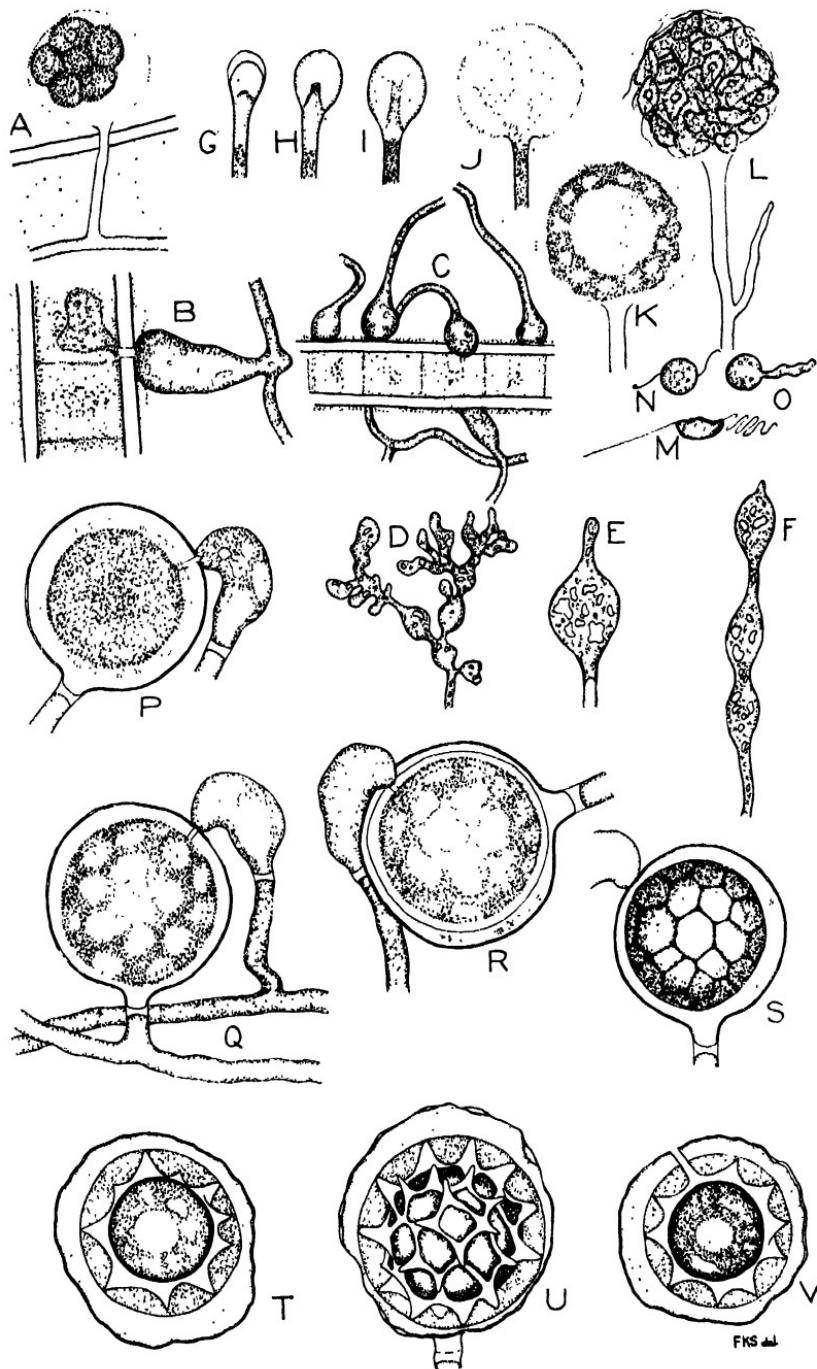
A brief consideration of the lack of sexual reproduction in pure culture, the identity of the various walls of the oöspore, the persistence of the fertilization tube, as well as a slightly emended technical description of the fungus, are included.

The writer wishes to express his appreciation for the aid and criticism given him by Professor W. H. Weston, Jr., under whose guidance the work reported in this paper was done.

LABORATORIES OF CRYPTOGAMIC BOTANY,  
HARVARD UNIVERSITY, CAMBRIDGE, MASS.

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#### EXPLANATION OF PLATE 20

All the illustrations are from living material except Text Fig. 1, A, B, and Plate 20, R, which are from material mounted in eosine and glycerine. The drawings were made with the aid of the camera lucida. The magnifications given refer to the original plates which, for purposes of publication, have been reduced approximately one-third.

Fig. A. Filamentous sporangium which has emerged through the cell wall of the *Spirogyra* and within the vesicle of which zoospores are being formed.  $\times 1500$ .

Fig. B. Appressorium attached to the wall of *Tolypothrix*, showing the penetration tube and the protoplasm of the fungus which has passed through this channel into the host cell.  $\times 2200$ .

Fig. C. A group of appressoria attached to a filament of *Tolypothrix*.  $\times 1400$ .

Figs. D, E, F. Hyphal swellings formed by the mycelium in protein media. Fig. D,  $\times 800$ ; Figs. E, F,  $\times 1400$ .

Figs. G, H, I, J. Stages in the formation of the vesicle and initiation of the egress of the sporangial protoplasm.  $\times 1400$ .

Fig. K. Vacuolate condition of the maturing protoplasm within the vesicle; characteristically found under foul conditions of environment.  $\times 1400$ .

Fig. L. Nearly mature zoospores within the vesicle.  $\times 1400$ .

Fig. M. Zoospore in side view.  $\times 1400$ .

Fig. N. Quiescent zoospore retracting its cilia and becoming modified into the cystospore.  $\times 1400$ .

Fig. O. Germinating cystospore.  $\times 1400$ .

Fig. P. Optical section of the fertilized egg, showing the differentiation of its content into periplasm and gonoplasm. The antheridial protoplasm may also be seen to be of two types.  $\times 2200$ .

Fig. Q. Fertilized egg showing the vacuolate periplasm.  $\times 2200$ .

Fig. R. A later stage in the development of the periplasm, showing the angular appearance of the vacuoles. Due to being mounted in glycerine, the contents of the egg have shrunk somewhat, revealing the thin periplasmic wall.  $\times 2200$ .

Fig. S. A still later stage in the maturation of the periplasm. The inter-vacuolar material has coalesced, forming the characteristic reticulations.  $\times 2200$ .

Fig. T. Optical section of the mature oöspore, showing the protoplasm surrounded by an innermost narrow endospore wall, a reticulate exospore wall and an outermost, thin periplasmic wall which encloses a gel-like substance.  $\times 2200$ .

Fig. U. Surface view of the mature oöspore showing the characteristic reticulations.  $\times 2200$ .

Fig. V. Optical section of a mature oöspore showing a persistent fertilization tube, which, in this case, was continuous with the exospore wall.  $\times 2200$ .

# A COMPARATIVE STUDY OF SCLEROTIUM ROLFSII AND SCLEROTIUM DELPHINII

F. L. STEVENS

(WITH 16 TEXT FIGURES)

In July, 1929, specimens of diseased *Delphinium* were received from Normal, McLean County, Illinois, with the statement that in 1928 it appeared only on species of *Delphinium*, but in 1929 on "anything that grows in the infested regions, regal lilies, golden banded lily of Japan, false dragon head, *Phlox*, *Iris*." Later letters also reported it on a rag weed, butter and eggs and pink and blue spider wort, *Verbena*, Madonna lily, tulips, *Funkia* lilies, blackberry lily, *Pyrethrum* daisy, yellow daisy, violets.

In September, 1929, specimens of diseased *Delphinium* root rot were received from Williamsville, Sangamon County, Illinois, with the report that the disease had been troublesome for two years in spots and was getting worse. Carrots were reported to have the same disease, during dry weather, but not in wet weather. The cause of the disease in the two instances reported above was clearly *S. Delphinii* Welch, but the two strains differed slightly. This fact and the obvious relation of this fungus to *Sclerotium Rolfsii* led to a somewhat extended comparison of these two organisms, which I designate respectively as *S. Delphinii* I and *S. Delphinii* II, with *Sclerotium Rolfsii* and with two strains of *S. Delphinii*, one an authentic culture, No. S 637 from Dr. Whetzel of Cornell, hereinafter designated merely as *S. Delphinii*, the other a culture from the University of Wisconsin which I designate as *S. Delphinii* III.

The *S. Rolfsii* here used was of the same character so far as I can judge as that which I sent to Professor Saccardo and which thus came to be the type of the species. This also is the same in character as the one that was received by Dr. Halsted from Professor Rolfs, which I personally studied and which is figured in *Plant Disease Fungi*, p. 438.

Professor Trotter kindly loaned me the type specimen from Saccardo's herbarium, but it is now entirely devoid of sclerotia and was dead, so that no cultures were obtainable.

I present first a description of this strain of *S. Rolfsii* as it usually appears on rice or carrots which may I think under the circumstances properly be regarded as an amplification of the type description and which may help to clarify the status as regards the numerous races of this fungus that have been reported. Dr. Higgins says that he has fifteen isolations which

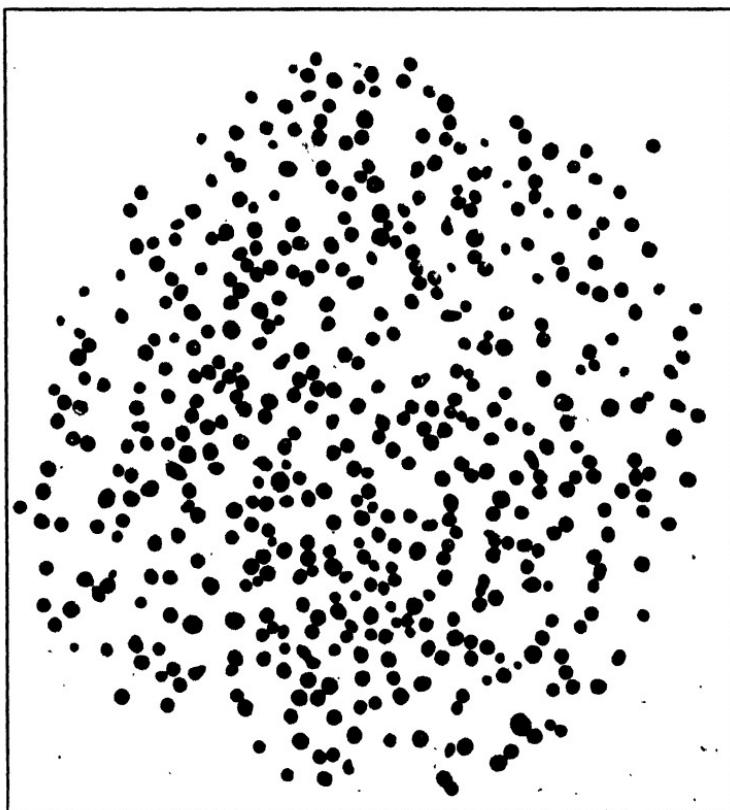


FIG. 1. Sclerotia of *S. Rolfsii* grown on carrot showing regularity in size and shape. See graph 1.

may probably represent five strains. Nakata<sup>1</sup> states that he has studied thirty-three strains. Edson and Shapovalov<sup>2</sup> report two strains.

<sup>1</sup> Nakata, K. Studies on *Sclerotium Rolfsii* Sacc. Part V. Physiological characters in relation to the strains of the fungus. Bull. Sc. Fac. Terkultara Kyushu Imp. Univ. 2: 237. 1927.

<sup>2</sup> Edson, H. A. & Shapovalov, N. Parasitism of *Sclerotium Rolfsii*. Jour. Agr. Res. 23: 41. 1923.

The sclerotia of *S. Rolfsii* are quite small, usually about 1.39 mm. in diameter (see also TABLE I), usually nearly or quite globose (FIG. 1), occasionally slightly irregular, and are very uniform in size. They arise in plexi formed in the aerial mycelium and with sufficient nutriment are very numerous, on corn-meal agar about 10 per square centimeter. They are usually borne singly but in rare instances two or more may coalesce. At first only a structureless white knot of tangled mycelium is seen without definite boundary or cortical differentiation. This soon assumes, one day, a globose form, definite boundary, and a cortical layer develops precisely as is the case with *S. Delphinii* described below. As these changes proceed the surface color changes first to pinkish buff, then to olive-brown and finally when old and dry to clove brown.<sup>3</sup> The surface is smooth, without markings, though liquid exudes as does from the sclerotia of *S. Delphinii*. The plexi which develop into sclerotia are borne on the aerial mycelium and the sclerotia are therefore usually free and suspended.

The sclerotia germinate by emitting numerous single mycelial threads between the cortical cells. The mycelium of the fungus consists either of long straight comparatively thick (7 mm.)

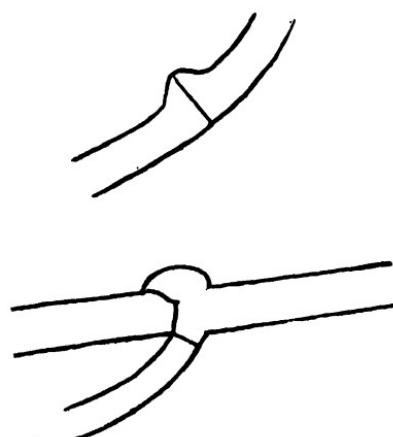


FIG. 2. *S. Rolfsii* showing clamp connections.

filaments, or much thinner and more crooked threads. The mycelium bears numerous typical clamp connections (FIG. 2).

<sup>3</sup> Colors from Ridgway standard.

The sclerotial plexi are composed of the more slender filaments. Always when sufficient humidity obtains there is a very dense development of aërial mycelium, floccose, wooly, often one to two centimeters in depth and so dense as to obscure vision of anything below it (FIG. 3).

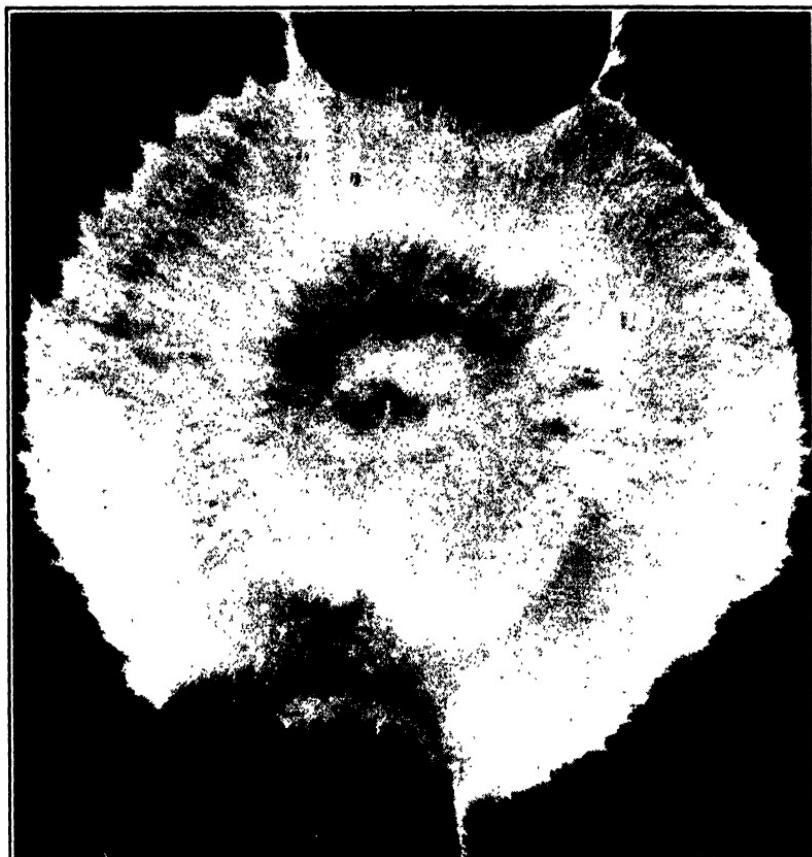


FIG. 3. *S. Rolfsii* growing on sterilized carrot, 5 days after inoculation, the aerial mycelium very floccose.  $\times 1\frac{1}{2}$ .

When *S. Rolfsii* was grown on corn-meal agar and the Petri dish inverted, it gave much floccose aërial mycelium reaching down to the Petri cover and producing numerous sclerotia suspended in air between the agar and the dish cover. Exceptions to the usual appearance of *S. Rolfsii* as given above are as follows:

*Sclerotium Rolfsii* when grown on rather poor nutrients as on tap water agar therefore with more scant development of mycelium than otherwise gives rise to ropy mycelial columns just as is the case with *S. Delphinii* and on these columns the sclerotia are borne. *S. Rolfsii*, which is usually characterized by its small regular sclerotia, under some conditions forms much larger sclerotia and these much less regular in form. *S. Rolfsii* sometimes germinates by a mycelial fascicle just as does *S. Delphinii*, and this may even bear a new sclerotium on its tip. Though ropy mycelium was usually not developed by *S. Rolfsii* this character was well developed on potato dextrose agar.

The description of *S. Delphinii* taken from the type culture received from Dr. Whetzel as it usually appears when growing on rice follows:

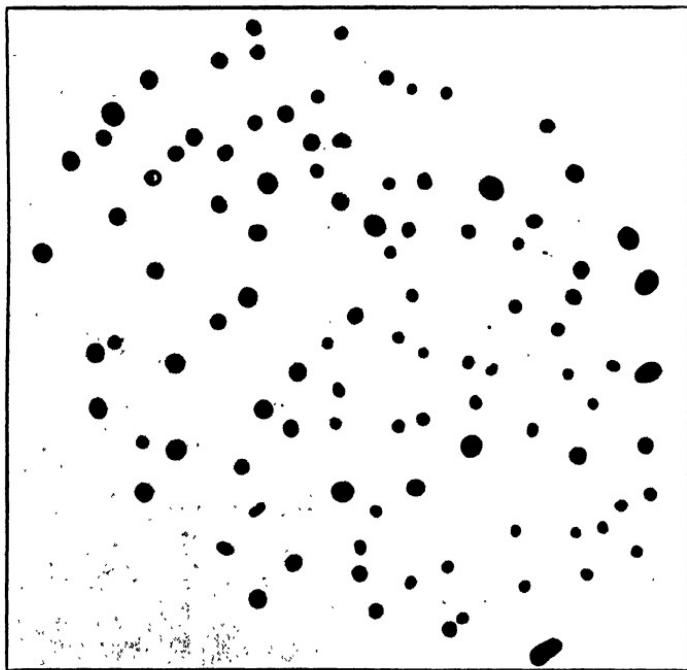


FIG. 4. Sclerotia of *S. Delphinii* as grown on corn-meal agar. See graph 2 for size.

The sclerotia vary greatly in size (FIG. 4), the mode being for the single sclerotia, 2.18 mm. in diameter, with a maximum of

5.18 mm. Irregular probably composite sclerotia may be 3.9  $\times$  9.8 mm. across. The single sclerotia are sub-globose, arched on top, but are hollowed below so that when inverted they are saucer-shaped and bear in the center a hilum-like scar, the place of attachment to the stalk that bore them. They often coalesce in considerable numbers, thus forming a crust of very irregular shape and size. The sclerotia are borne on the apices of mycelial columns (FIGS. 5, 6, 7) which are from 0.5-3 mm. thick and 2-5 mm. tall, which arise from the substratum and which are composed of very numerous fascicled mycelial threads running parallel. The sclerotia first appear as a white globose enlarge-

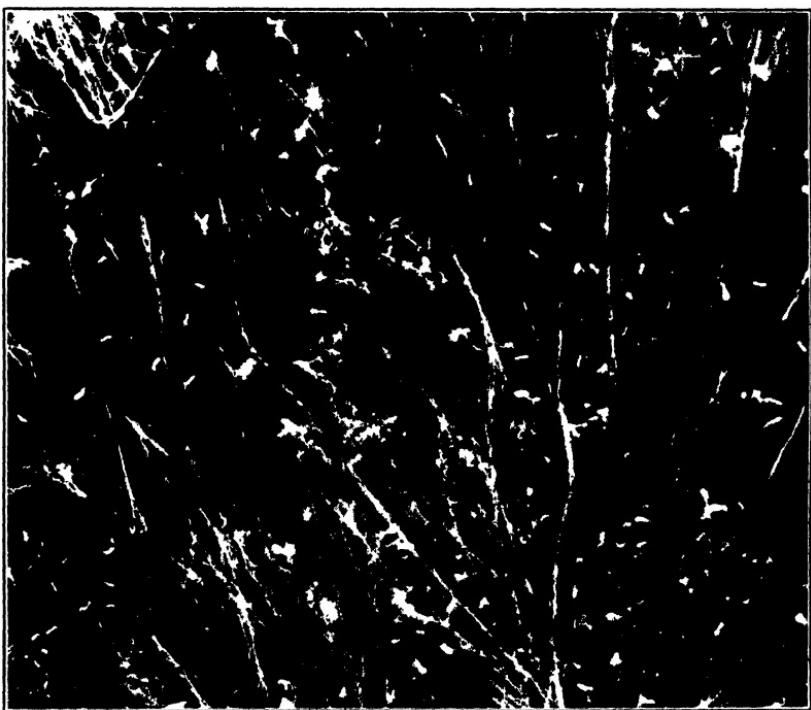


FIG. 5. *S. Delphinii* showing the sclerotia borne on stalks.

ment at the apex of the mycelial fascicle. Later they enlarge much in lateral diameter though but slightly in depth. At first they are without definite boundary or cortex, but they soon become of even surface and a cortex one cell thick develops. *S. Delphinii* killed six days after inoculation while the sclerotia

were still white showed the sclerotia globular and well delimited at the surface. A sclerotium 1.386 mm. in diameter possessed a very definite cortical layer about  $30\ \mu$  thick. This cortex is composed of short, thick, irregular cells so intimately in contact with each other that no intercellular spaces appear. All of the region of the sclerotium within the cortex is uniform in character and consists of cells of very irregular shape, some long, some short and with many intercellular cavities. There is a larger proportion of long cells than is the case with *S. Rolfsii* (FIG. 8). If the sclerotia enlarge to over about 1.5 mm. in diameter they exude clear liquid at numerous points on the surface. The points of exudation appear different in color from the surrounding cortex. They vary in size from  $540\ \mu$  in diameter downward to mere points and when large remain distinctly visible on old dried sclerotia (FIG. 9). Fresh sclerotia in section show the region immediately under these spots to have a water soaked appearance which extends downward into the medulla to a depth about equal to the diameter of the spot, but no morphological difference in them is discernible.



FIG. 6. *S. Delphinii* showing the stalks of the sclerotia.

The sclerotia as they age pass to ochraceous-buff, then to tawny and finally to Hay's brown.<sup>4</sup> The sclerotia usually germinate by emitting mycelial sheaves from the surface spots, these sheaves being very like those that bear sclerotia. Indeed, if conditions do not favor continued growth each sheaf may immediately terminate in a new sclerotium, so that in some instances a large sclerotium may bear twenty or more mycelial sheaves each with a new sclerotium at its tip. Such sclerotia are globose, smooth, unmarked and smaller than the usual sclerotium of *S. Rolfsii*.

The mycelial threads are quite like those of *S. Rolfsii* except

<sup>4</sup> Colors from Ridgway standard.

that the coarser threads are a trifle thinner,  $5.4 \mu$ , and in usual conditions they aggregate into ropy dendritic strands, Figure 10, which are composed of very numerous individual filaments. There is almost entire absence of aerial mycelium. When *S. Delphinii* was grown on corn-meal agar and the Petri dish inverted, it made no aerial mycelium or very little and that little was aggregated into mycelial ropes. The mycelium bears numerous typical clamp connections (FIG. 11). In this as in the other strains they are most easily demonstrated by growing the fungus on potato dextrose agar in inverted plates and examining the aerial mycelium.

Exceptions to the usual appearance of *S. Delphinii* as given above are as follows: The surface liquid exudation and attendant



FIG. 7. *S. Delphinii* showing a single sclerotium on its stalk.

surface marking so almost universal in this species are much less pronounced in some conditions and quite absent in others, for example, in a dry atmosphere or when sclerotia are so poorly nourished as to remain small. In the absence of suitable nutriment as on corn-meal agar and especially on tap water agar sclerotia remain very small (see TABLE I), uniform in size and regular.

There are several important characters that usually, at a glance, serve to separate *S. Rolfsii* from the four strains of *S. Delphinii*. First among these is the absence in *S. Rolfsii* of a stalk to the sclerotia. These, however, are not always absent and in some cases appear quite as typical as in the case of *S. Delphinii*.

Second is the surface marking of the sclerotium. This is never seen in *S. Rolfsii* and is usually present in *S. Delphinii*. However, if *S. Delphinii* is cultured in such poor nutriment as to

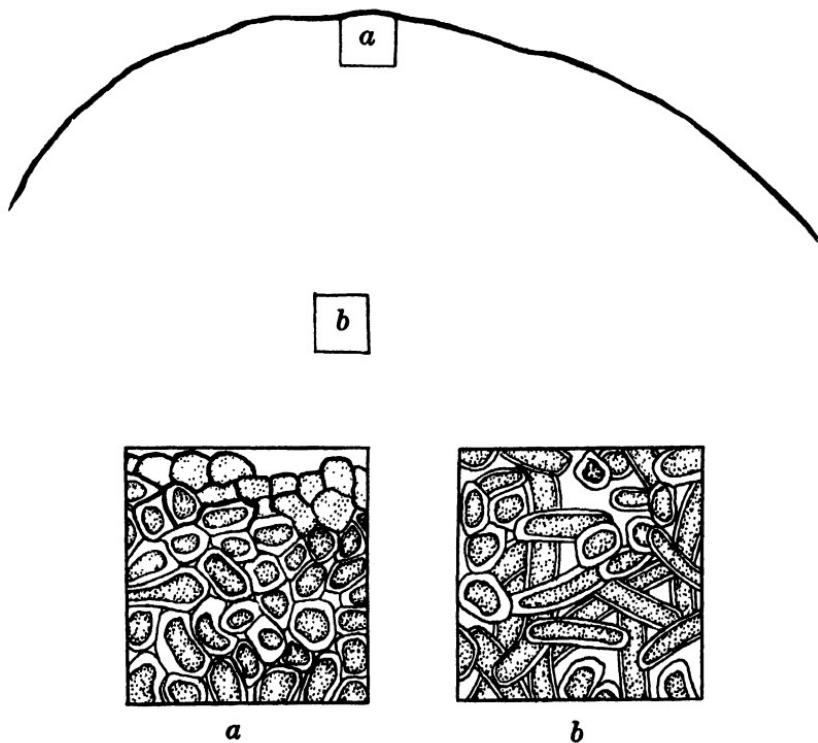


FIG. 8. *S. Delphinii*. Section of sclerotium; *a*, showing cortex, *b*, showing deeper region.

give only small sclerotia no pits develop. The small uniform sclerotia of *S. Rolfsii* contrast strongly with the large irregular sclerotia of *S. Delphinii*. Yet with absence of proper nutrients *S. Delphinii* forms sclerotia that are small and quite uniform in size, while on suitable nutrients *S. Rolfsii* forms sclerotia much larger, more irregular in size than usual, also more irregular in

shape. Germination of the *S. Delphinii* type of sclerotium is by the emergence of a dense fascicle of mycelium from one of the surface marks while *S. Rolfsii* usually germinates by single mycelial threads. In some cases *S. Rolfsii*, however, is seen to send forth a fascicle and this may even bear a new sclerotium on its tip.

*S. Rolfsii* usually shows an abundant floccose aerial mycelium, not ropy, while that of *S. Delphinii* is not floccose but is ropy. Under some conditions, however, *S. Rolfsii* assumes the ropy form though *S. Delphinii* never becomes floccose. The sclerotia of *S. Rolfsii* are never saucer shaped below, those of *S. Delphinii* are, but when poorly nourished *S. Delphinii* produces small sclerotia that do not show this character. *S. Rolfsii* makes very numerous sclerotia; *S. Delphinii* much fewer, but this difference is removed under certain conditions. *S. Rolfsii* and *S. Delphinii* so far as I have seen always differ in color of mature sclerotia as has been indicated above. Thus it is seen that while readily separable, if conditions are favorable for separation, these two forms lose nearly all of their differentiating characters under certain conditions.

Tabulated this appears as follows:

	<i>S. Rolfsii</i>	<i>S. Delphinii</i>
Mycelium . . . . .	Densely floccose † Not ropy †	Not floccose * Ropy *
Sclerotia . . . . .	Not concave * Not surface marked * Color pinkish buff to olive brown to clove brown Very numerous † Small *	Concave † Surface marked † Color ochraceous buff to tawny to Hay's brown Less numerous † Larger †
Germination . . . . .	Single threads †	Fascicled †

A \* indicates that the character is constant; a † that it is not.

The only really differentiating character in all conditions is sclerotial color.

The four races of *S. Delphinii* are very closely alike though in certain conditions they show such differences that they must be regarded as four distinct strains. For example it is found that when inoculations of two of these races are made upon corn-

meal agar in one Petri dish the two colonies grow toward each other until they nearly meet. Then at the line of junction of the two a band, very distinct to the naked eye, about 3 mm. wide, and extending completely across the plate, is seen. Microscopically this line is seen to be caused by a very abnormal profuse irregular branching together with many smaller cells where the two colonies meet. Such a band was produced whenever any one of the five races considered above was grown on the same dish with any other of the five races.

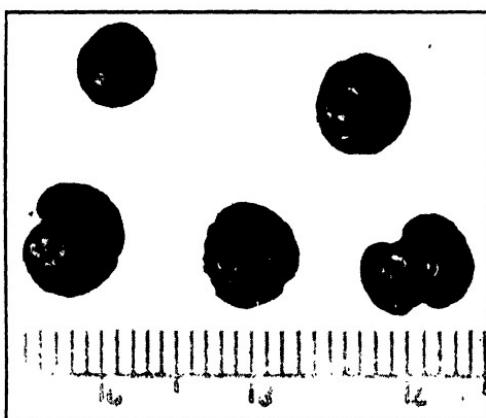


FIG. 9. Sclerotia of *S. Delphinii* enlarged mush, showing surface markings.

When any races were grown against another colony of the same race the mycelium of the two colonies mingled freely and no abnormal branching resulted. In none of the six oppositions was any consistent influence upon the number of sclerotia formed observed though occasionally sclerotia were more abundant where two colonies met, nor did most close search reveal any spore forms. Taubenhaus<sup>5</sup> stated that in his experiments sclerotia were more numerous where colonies met which he interprets as due to the existence of plus and minus strains. This appears to me extremely doubtful.

It seems probable though it is difficult to determine with certainty that when *S. Delphinii* II and *S. Rolfsii* are planted

<sup>5</sup> Taubenhaus, J. T. Recent studies on *Sclerotium Rolfsii* Sacc. Jour. Agr. Res. 18: 127. 1919.

together the profuse branching is of the *S. Delphinii* II colony. When *S. Delphinii* I and *S. Rolfsii* come together the abnormal branching is of the *S. Rolfsii* colony.

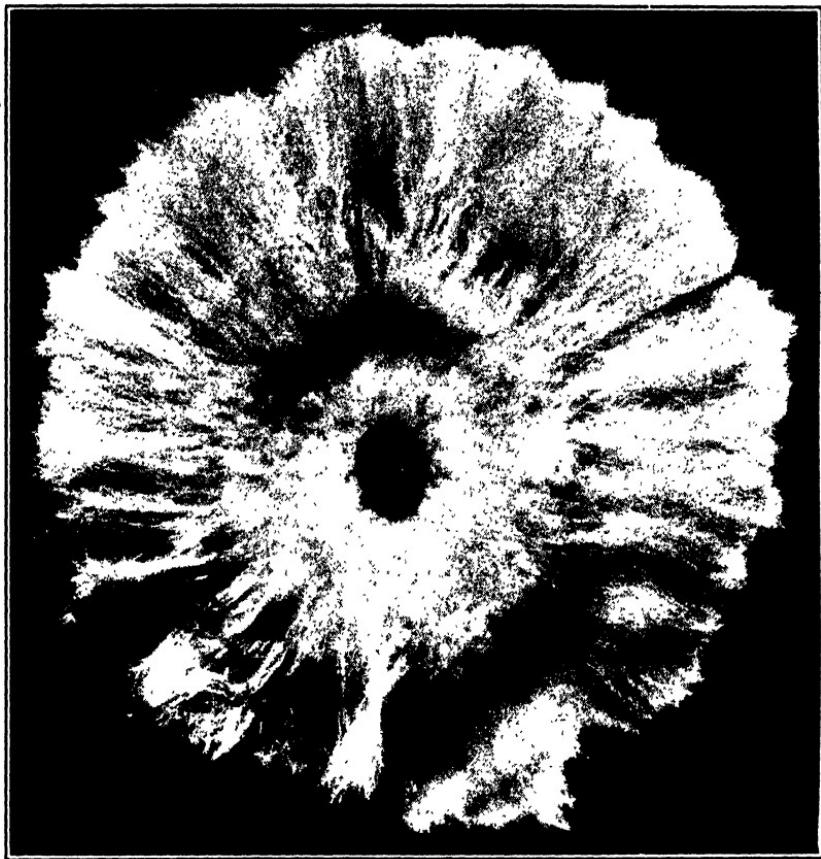


FIG. 10. *S. Delphinii* five days after inoculation, dendritic, not fluffy,  $\times 1\frac{1}{2}$ .

It appears therefore that *S. Rolfsii* exerts a repellent antagonistic effect on *S. Delphinii* II and that *S. Delphinii* I exerts a similar effect upon *S. Rolfsii*. This antagonism is of the class discussed by Porter<sup>6</sup> and indicates here a difference between the strains showing it and does not appear when like strains were planted together. The five races were grown on various media

<sup>6</sup> Porter, C. L. Concerning the characters of certain fungi as exhibited by their growth in the presence of other fungi. Amer. Jour. Bot. 11: 168. 1924.

TABLE I  
SHOWING THE SIZE OF SCLEROTIA OF VARIOUS RACES ON VARIOUS MEDIA EXPRESSED IN MILLIMETERS OF DIAMETER

	<i>S. Radfpii</i>	<i>S. Delphinii</i>	<i>S. Delphinii</i> III	<i>S. Delphinii</i> I	<i>S. Delphinii</i> II
On steamed rice					
mode.....	2.189	2.189		3.283	2.189
max.....	5.174	4.975		6.567	6.567
min.....	1.691	1.691		1.691	1.094
On second reading.....				3.223	
				6.268	
				1.592	
On carrot					
mode.....	1.393				
max.....	2.985	see Fig. 1			
min.....	.950				
Tap water agar					
mode.....	.995	.895		.895	.696
max.....	1.194	1.293		1.393	1.791
min.....	.574	.796		.597	.696
Halved orange					
mode.....	1.791			3.183	
max.....	2.686			5.472	
min.....	1.393			2.189	
On corn-meal agar					
mode.....	1.791	1.592		1.393	
max.....	3.383	2.487		2.487	
min.....	1.194	1.194		1.194	
Potato dextrose agar					
mode.....				2.189	
max.....				4.975	
min.....				1.891	
Mush					
mode.....	2.189			3.283	
max.....	4.676			6.567	
min.....	1.691			2.189	

and measurements of the sclerotia were made and are recorded below.



FIG. 11. *S. Delphinii* III clamp connections.

In addition to the sclerotia represented above there were in some cases more or less numerous, very irregular sclerotia, in some cases composites by coalescence of several, which could not with fairness be represented in the general tabulation. On rice in the case of *S. Delphinii* there were 16 such ranging from 2189 to 6069 wide by 3.880 to 9850  $\mu$  long.

*S. Delphinii* III gave 22 of these, width 2.189–5.372  $\times$  4.378–8.756  $\mu$ .

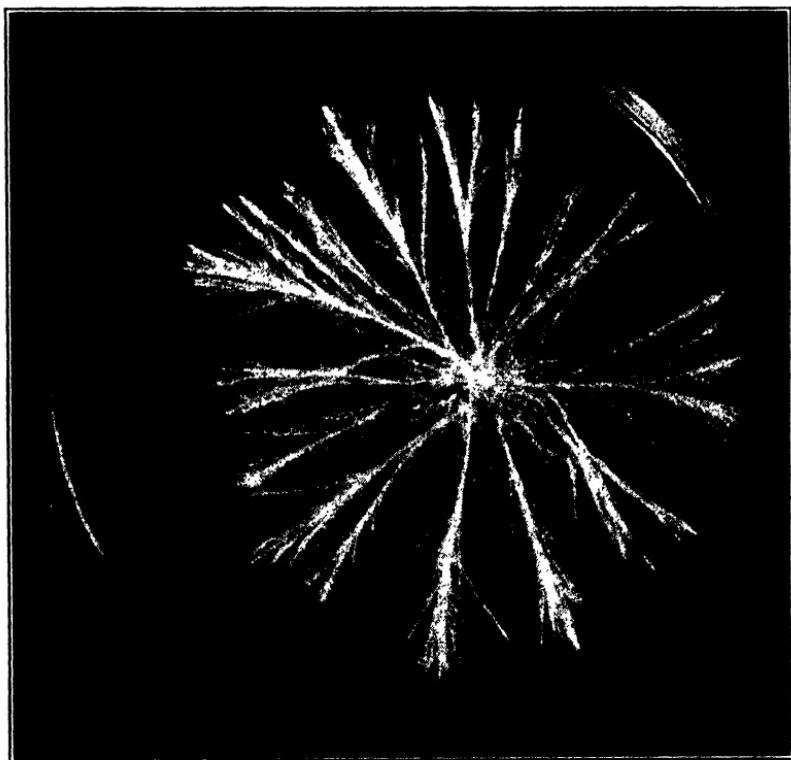


FIG. 12. *S. Delphinii* II on corn-meal agar showing dendritic growth.

*S. Delphinii* I gave 19 of these, width 2.189–5.870 × 5.372–9.850  $\mu$ .

*S. Delphinii* II gave 8 of these, width 2.786–5.372 × 5.372–9.850  $\mu$ .

On tap water agar, *i.e.*, without any added nutrients, growth was scanty with all five races and very few sclerotia were formed, more by *S. Rolfsii* than by any other race. The sclerotia in all cases were small. The modal differences in the tables are not significant because the sclerotia occurred in too small numbers, though it is significant that all sclerotia were very small, also that all were devoid of surface pitting. Graphs showing the sclerotial size as growing on rice are given in Figure 13. There is close agreement here between *S. Delphinii* and *S. Delphinii* III and *S. Delphinii* II but *S. Delphinii* I seems to differ.

*S. Rolfsii* was graphed only on carrot because it was usually so uniform in the size of its sclerotia.

An increase in size of sclerotia is shown by *S. Rolfsii* on corn mush over that on any other medium tried. On orange there was slight increase in the mode over that on carrot. Corn-meal agar shows its inferior nutrient value with the races of *S. Delphinii* in that the sclerotia are distinctly smaller than when these races are grown on rice. Potato dextrose agar, however, was equal to rice in value for *S. Delphinii* III as was also mush for *S. Delphinii* I. The carbohydrate here is probably the needed food.

All five races were grown on potato dextrose agar and irradiated when the colonies were 6 cm. in diameter with ultra-violet for various dosages. All showed a distinct stunting effect with 30 seconds' irradiation, and some stunting with five seconds, and sclerotial formation was permanently inhibited on the irradiated area (FIG. 14), though they did form on the new growth arising from the irradiated area. One day after irradiation a distinct band of aerial mycelium about 4 mm. wide was visible at the locus of the irradiated mycelial tips in *S. Delphinii* II and *S. Delphinii* I.

Dr. Nakata very kindly sent me six strains of *S. Rolfsii* studied by him, namely No. 501 isolated from potato at Kyushi, Uni-

versity Agriculture Farm, No. 502, from petunia at Kyushi, No. 519, from carrot at Kyushi, No. 524, from Konjac (*Amorphophallus*) at Kyushi, No. 527, from sugar beet in Korea, No. 509, from Konjac at Takushima.

While I am unable to make extensive comparisons of these with my strains the following notes may be worth publishing:

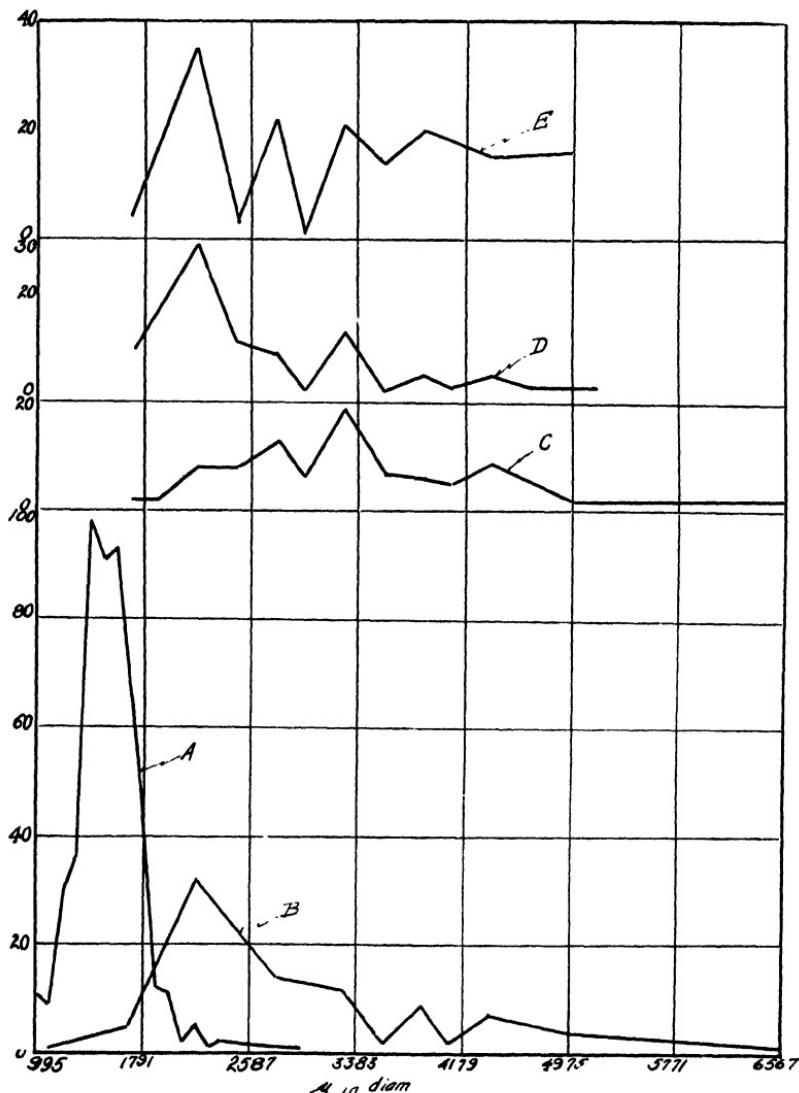


FIG. 13. Graphs showing sclerotial size of five strains of *Sclerotium*. A, *S. Roflsii*; B, *S. Delphinii* II; C, *S. Delphinii* I; D, *S. Delphinii*; E, *S. Delphinii* III.

## ON CORN-MEAL AGAR

- No. 501. Very like *S. Rolfsii*, sclerotia a trifle larger.  
No. 502. As above.  
No. 519. Sclerotia a trifle smaller than above numbers and frequently showing stipes.  
No. 524. As in No. 501.  
No. 527. Sclerotia twice the diameter of those of No. 501 and few and showing surface spots, stipes common.  
No. 569. As in No. 501.

## ON POTATO DEXTROSE AGAR

- No. 501. Much as on corn-meal agar but growth more luxuriant.  
No. 502. As above, but of very different growth habit.  
No. 519. Sclerotia very few.  
No. 524. Sclerotia very few, but habit different from that of No. 519.  
No. 527. Growth habit very different from all others.  
No. 569. Much like No. 501 but with much fewer sclerotia.

Comparison of the results on these two media gives conclusive evidence that these six strains show considerable differences and since in none of these was there the abundant aerial mycelium so characteristic of my *S. Rolfsii*; they also differ from the five cultures that I am studying.

To ascertain the relative rapidity of migration through soils of various degrees of wetness glass tubes 13 mm. inside diameter were cut 5 cm. long and fitted at each end with corks each bearing four longitudinal grooves to allow exchange of air and moisture. These tubes were filled with soil and water added 1, 2, 3 and 0 cc. per tube. Three cubic centimeters of water made the soil very wet. Each tube was inoculated with *S. Rolfsii* Jan. 20 by placing a mass of mycelium under the cork at one end. The tubes were then placed on glass benches in a moist chamber. The photograph Fig. 15 was taken Jan. 24, showing growth in direct relation to the amount of water present. At seven days from inoculation the soil that received no water showed no growth of the mycelium; that with 1 cc. and 2 cc. had advanced 17 mm. each while that with 3 cc. had advanced 4 cm.

To determine the rate of growth in soils of different qualities

tubes like those described above were used with each of the following: rich loam, horse manure, sand, soil and manure and soil and sand, and tests made of rate of lineal growth through the



FIG. 14. *Sclerotium Delphinii* showing complete suppression of sclerotia by 30" ultra-violet irradiation.

tube. Four tubes of each composition were inoculated separately with each of the five strains and half of them kept in the dark, the others in the light. The results are given in the following table with the average lineal growth expressed in millimeters.

TABLE II

	<i>S. Delphinii</i>	<i>S. Delphinii III</i>	<i>S. Delphinii I</i>	<i>S. Delphinii II</i>	<i>S. Rolfsii</i>
1. Loam . . . . .	45 mm.	45	45	45	45
2. Manure. . . . .	24 mm.	12	22	12	15
3. Sand . . . . .	25 mm.	25	23	12	45
4. Soil and ma- nure. . . . .	45 mm.	45	45	45	45
5. Soil and sand	12 mm.	17	30	8	45

It is seen that growth is most rapid in loam or loam and manure and that it is about equal for the five races. The growth in manure was very poor as was also the growth in sand or sand and loam, with the exception however that *S. Rolfsii* grew at its maximum in each tube except that containing the manure.

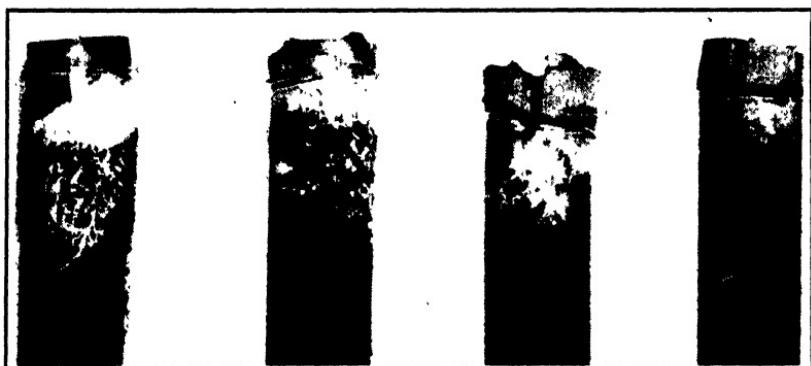


FIG. 15. Showing relative rate of growth of *S. Rolfsii* in soils of different water content; from left to right was added 0, 1, 2, 3 cc. of water.

The experiment was repeated using each of the soils mentioned above but employing two series of soils, one loosely filled in, the other tamped in. No difference whatever was noticeable between the growth in the tamped and the loose soil.

King and Loomis,<sup>7</sup> who made somewhat similar experiments, state that "After trial of various types of soil, it was found that the mycelial strands grow most rapidly in pure sand."

From all of the facts it appears that all of the races here considered are very closely related and might indeed be considered merely as varieties of one species, though the considerable differences presented between *S. Rolfsii* and *S. Delphinii* may warrant the retention of the latter as a separate species.

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<sup>7</sup> King, C. J., and Loomis, H. F. Further studies of cotton root rot in Arizona with a description of a sclerotium stage of the fungus. Jour. Agr. Res. 39: 665. 1929.

## GLOEOSPORIUM ROSAE, A NOMEN NUDUM

ANNA E. JENKINS

*Gloeosporium Rosae* Hals. (3) causing a destructive rose (*Rosa*) disease, termed anthracnose (3), is apparently to be regarded as a *nomen nudum*. The disease was considered by Halsted as possibly identical with anthracnose of brambles (*Rubus*). As discussed by him, however, practically all of its etiological and symptomatological features (3, 4, 5) are unlike those of the latter distinctive disease. In the main they suggest a disease more like those caused by Sphaeriales, such as *Glomerella* or *Diaporthe*. Insufficient data were given by Halsted even to establish the generic identity of the organism to which the name *Gloeosporium Rosae* was applied. Through correspondence with Elizabeth Clark, New Jersey Experiment Station, it has been learned that the specimens studied by Halsted are not available there. None were found by the writer in the Mycological Herbarium of The New York Botanical Garden and in that at the Bureau of Plant Industry. Through Dr. W. H. Weston it has been learned that such specimens are not to be found in the Farlow Herbarium at Harvard University. Thus no basis has been found on which to establish the identity of the fungus associated with Halsted's rose anthracnose. The binomial *Gloeosporium Rosae* is not mentioned in such standard mycological works as Saccardo's *Sylloge Fungorum*. It is found in a number of references pathological in character. In some of them its mention is based directly upon data presented by Halsted, although in one instance (1: 92) it represents material of which a part was recently recognized by the writer as *Diaporthe umbrina* Jenkins. Seymour's (9: 396) listing of *Glomerella cincta* (Stoneman) Spaulding and von Schrenk as the perfect stage of *Gloeosporium Rosae* is based upon publication to this effect by Schwartze (8: 58).<sup>1</sup>

Reference (2) to anthracnose "affecting the raspberry, blackberry and rose" probably has a direct relation to Halsted's

<sup>1</sup> Information from the files of A. B. Seymour, contributed by C. W. Dodge.

suggestion that the anthracnose of brambles and his rose anthracnose may be identical diseases. Since it is not known to what rose disease Halsted applied the term anthracnose, that name as so employed by him is now without significance. It has been explained previously (6) that Sheldon (10) may have discussed under the title of "Some rose anthracnoses" the rose disease now known as brown canker. That other rose diseases which he (10) referred to at the same time are not definitely diagnosed has been learned through correspondence or personal conference with Professor Sheldon.

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## AMANITA CALYPTRATA AND AMANITA CALYPTRODERMA

S. M. ZELLER

In January, 1900, Peck<sup>1</sup> described *Amanita calyptata* Peck from Oregon material sent in by Dr. H. Lane during the autumn. Another Pacific Coast species closely related to the latter was described in 1909. It is very interesting but not strange that two great American mycologists, George F. Atkinson and Charles H. Peck, should within six days publish descriptions of and give similar names to this *Amanita*. Both received material from California. Atkinson received material during the fall and winter of 1908 from Mrs. V. G. Ballen, of Brookdale, Santa Cruz County. Mrs. Ballen provided such adequate, distinguishing notes, photographs and specimens that it was published jointly as *Amanita calyptroderma* Atkinson & Ballen. This description was published first in Science, June 11, 1909.<sup>2</sup> Only six days later was issued Peck's description of the same plant under the name *Amanita calyptatoidea* Peck.<sup>3</sup> Peck's name refers to the similarity of this plant to his previously described *A. calyptata*, while Atkinson and Ballen use the name *A. calyptroderma* "because the calyptra of the volva fits like a skin over the center of the pileus."

A description of the local distribution in California, ecology and field notes on *A. calyptroderma* appeared later the same year.<sup>4</sup> Herein is mentioned the close relationship of this species with the more robust European *A. caesarea* and Peck's *A. calyptata*.

Murrill<sup>5</sup> and Kauffman<sup>6</sup> have given preference to Peck's

<sup>1</sup> Peck, Chas. H. New species of fungi. Bull. Torrey Club **27**: 14. 1900.

<sup>2</sup> Atkinson, G. F. A new edible species of Amanita. Science, N. S. **29**: 944. June 11, 1909.

<sup>3</sup> Peck, C. H. New species of fungi. Bull. Torrey Club **36**: 329-330. June 17, 1909.

<sup>4</sup> Atkinson, G. F. A remarkable *Amanita*. Bot. Gaz. **48**: 283-293. Illus. Oct. 1909.

<sup>5</sup> Murrill, W. A. Agaricaceae. *Venenarius*. N. Am. Fl. **10**: 71; 75. 1914.

<sup>6</sup> Kauffman, C. H. Fungous flora of the Siskiyou Mountains in southern Oregon. Mich. Acad. Sci., Arts, and Letters, Papers **11**: 180. 1930.

name because they evidently overlooked Atkinson's description in Science, but they have referred doubtless to a description published later by Atkinson.<sup>7</sup> Murrill gives to *A. calyptrotrata* Peck the new name *Venenarius Lanei* because of the pre-Friesian *Amanita calyptrotrata* Lam. 1778. Kauffman evidently had not seen Atkinson's<sup>4</sup> discussion and description of the double cup at the base of *A. calyptroderma*.

These two species, *A. calyptrotrata* Peck and *A. calyptroderma* Atkinson and Ballen, are very similar and difficult to distinguish. The main distinction is the greenish tinting of the pileus and gills in *A. calyptrotrata*, and the thick double volvate cup at the base of the stem in *A. calyptroderma*. Fortunately, both species are edible. The Italians consume many of them and very likely presume them to be identical with their old acquaintance, *A. Caesarea*, of their native land.

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<sup>7</sup> Atkinson, Geo. F. Preliminary notes on some new species of Agaricaceae and Clavaria. Ann. Myc. 7: 365. August, 1909.

# HERBARIUM ARRANGEMENT OF MYCOLOGICAL SPECIMENS<sup>1, 2</sup>

E. T. BARTHOLOMEW

(WITH PLATES 21 AND 22 AND 4 TEXT FIGURES)

How shall the specimens be arranged so that they will be most readily accessible and at the same time be best preserved, is one of the questions that presents itself to every herbarium curator who wishes to build up an efficient mycological herbarium. The same question also arises when it becomes advisable to rearrange the specimens in an already existing herbarium.

A mycological herbarium to be of the greatest value to those who wish to use it should have its specimens so cared for that they will continue to remain as nearly as possible in the original state of preservation, and it must be workable. It should have the information which it contains as logically arranged and as readily available as the information in a dictionary or an encyclopedia. Probably this is an ideal which has never been actually attained, but more nearly so in some herbaria than in others. However, it has been the personal observation of the writer, and his observation has been corroborated through correspondence, that many, if not most, of the mycological herbaria in the United States are very much below par in their efficiency.

So far as the writer has been able to ascertain, the American literature contains no detailed discussions of this subject. It is for this reason that the methods of arrangement in use in some of the larger mycological herbaria in the United States are being described and discussed in this paper.

<sup>1</sup> Paper No. 224, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

<sup>2</sup> It is with pleasure that the writer acknowledges the receipt of data and samples from numerous herbarium curators, and especially the helpful criticisms by Dr. H. M. Fitzpatrick of Cornell University, Dr. H. S. Jackson of the University of Toronto, and Dr. H. D. House of the New York State Museum, who read the manuscript.

## METHODS OF FILING MYCOLOGICAL SPECIMENS

## 1. Specimens in Packets Attached to Herbarium Sheets

Flat, thin mycological specimens, for example, such as are found in the Phycomycetes, Ascomycetes and Basidiomycetes, are placed in specially-folded envelopes called packets.<sup>4</sup> In the majority of the herbaria these packets are attached to sheets of heavy, white paper, approximately 11 × 17 inches in size. This

No.	Name of Herbarium
S.Pkt.	.....
L.Pkt.	Hab.....
Liquid	.....
Box	Loc.....
Ill.	.....
Culture	Leg.....
Slide	Date.....
Paraf.	.....
Neg.	Source.....
Photo.	Det.....
Lant.Sl.	.....
Letter	Notes
Notes	
Dupl.	

FIG. 1. These or similar data will be found helpful if printed on the card that is to be used in the accession or species card-index file of the herbarium.

is the standard herbarium sheet used in mounting phanerogamic specimens. However, in some herbaria a sheet only half that size, 8½ × 11 inches, is used. The packets are either glued or pinned to these sheets. If the latter method has been used the packets may be transferred to a different sheet with less danger of marring and making unsightly the one to which it was originally attached. Such transfers are desirable when, for example, it has been decided to change to a different system of classification. The size of the packet varies, but for the sake of conservation of space it conforms as nearly as possible to the size of the specimen which it contains.

<sup>4</sup> Perhaps it would be more correct to use the word "pocket" or "envelope" when referring to the folded receptacle alone and the word "packet" when referring to the folded receptacle containing a specimen, but for the sake of uniformity and to follow common usage only the latter will be used.

The large sheets, each bearing one or more packets, are put into folders of heavy manila paper and laid flat, one on top of another, in the herbarium pigeon hole or compartment. A separate folder may be used for each genus, for each species or for each species on a given host. The smaller-sized sheets may be filed in the same manner or, as in some herbaria, they may be placed in folders of proper size and filed vertically in letter-file fashion.

In some herbaria it is desired to indicate geographical distribution from different parts of the world without resorting to a complete segregation. This is done by having a different colored folder for different countries or groups of countries. For example, for collections from North America the folders may be the common manila, from Europe they may be blue, and from the Asiatic regions white. Folders for the collections from the local state may be of still another color. While such a plan would require more space than if all species of a given genus were filed regardless of distribution, it would probably require less space than if the different collections were segregated and kept in separate parts of the herbarium.

The following are a few of the herbaria in which all but the bulky specimens are placed in packets and attached to herbarium sheets: United States Herbarium in Washington, the Farlow Herbarium at Harvard, the Arthur Herbarium at Purdue, the Atkinson Herbarium at Cornell, The New York Botanical Garden Herbarium and the Missouri Botanical Garden Herbarium. In all of these the herbarium sheets are of standard size and are filed flat. At the University of Wisconsin, Iowa State Agricultural College, Brooklyn Botanic Garden, and in some private herbaria the packets are attached to sheets that are only one-half the standard size. In the two former they are filed flat and in the latter usually vertically.

**ADVANTAGES OF METHOD.**—By filing in this manner, all (but not an individual) of the species of a given genus or of a given host may be available with a minimum of time and labor; an entire infected host plant may be preserved; drawings or photographs of the fungus may be attached to the sheet beside the packet; the letter or separate containing the original description

of the species may be attached to the sheet which bears it; and the specimens lie flat and do not drop to the bottom of the packet, unless the sheets are filed vertically, as they are in a few instances where the sheets are only half size. For an example of how the packets, drawings and notes may be placed side by side on the same herbarium sheet see plate 21.<sup>5</sup> Perhaps one of the greatest advantages of this system lies in the fact that where the specimens are filed flat, herbarium cases of the same size and design may be used for all specimens, no matter what their size. Practically no specimens need to be reduced in size. The large ones, such as appear in the Basidiomycetes and Gasteromycetes, may be placed in boxes and filed in the same compartment as the folder which contains the flat specimens of the same genus or species. This method also conserves the time of the curator in that usually the original packet and label are retained and all that is necessary for filing is to pin or glue the packet to the proper sheet.

**DISADVANTAGES.**—In order to examine an individual specimen the folder must be taken from the cabinet compartment, a suitable table space on which to open the folder must be located, and then several sheets may have to be shifted in order to find the desired specimen. In the hands of an expert the packet sheets can be handled or shifted without harm but a careless worker or the person who has had a minimum of experience in herbarium work may do much injury to fragile specimens by permitting the large sheets to bend excessively while he is handling them. This excessive bending may also release the fold at the end of the packet. Time must be taken to rearrange the fold or the packet sheet looks untidy; furthermore, if this is not done, portions of the specimen may protrude or even be lost from the packet. A comparatively large space is required in which to file specimens in this manner. Within the herbarium there may be a large number of the sheets that temporarily bear only one packet, thus requiring not only excessive filing space, but filing material. If the packet sheets are only half size and are filed vertically in the drawers of a letter file a stack of four drawers

<sup>5</sup> The photograph for this plate was kindly sent to me by Dr. J. C. Arthur of Purdue University.

is the most that can be used without requiring the use of a step ladder. Such an arrangement is not conservative of floor space. This plan is also subject to the objection that the specimen drops to the bottom of the packet and that there is more danger of crowding and breakage than if the sheets are laid horizontally. Specimens might be injured by accidentally inserting one folder inside of another when replacing a folder in the drawer. This is an objection which has been given, but it does not seem probable that it is an important one. Where packets of varying sizes are used a certain amount of space on the packet sheet cannot be utilized, and this is especially true when the half-size sheet is used.

## *2. Specimens Filed Vertically in Packets or Envelopes*

In some herbaria the packets are not attached to herbarium sheets, but are filed vertically in boxes or drawers, like cards in a library card index system, with appropriate guide cards. In some cases the packets are filed just as they are, while in other cases each packet is first placed in an envelope and then filed. In the former, packets of a uniform size are used; in the latter they may be of a uniform size or there may be a certain amount of variation in the size of the packet, but the envelopes are all of a standard size. The size of the packet or envelope may vary with the herbarium, or more than one size may be used in the same herbarium.

In some herbaria all packets are put into a standard  $3\frac{1}{2} \times 6$  inch envelope with a non-gummed flap. These envelopes are made of about twenty-pound manila paper, or of paper of similar weight and quality but of some other color. The latter are used where it is desired to designate specimens from a given locality without having to resort to segregation.

Where the packets are filed without the use of an envelope the same results may be obtained by using paper of different colors from which to make the packets. A similar result is also obtained in some herbaria where ink of different colors is used in typing or writing the data on the outside of the packet or envelope.

In at least one herbarium the packets are all uniform in size

( $5 \times 6\frac{1}{2}$  inches) and then these are placed in a  $5\frac{1}{2} \times 7\frac{1}{4}$  modified photographer's envelope. These envelopes contain the usual thumb and finger "cut-out" at the open end but the legend is written parallel with the long rather than with the short edge and the envelopes are filed accordingly.

In different herbaria where this system of filing is followed the packet or envelope appears to vary in size from  $3 \times 5$  to  $5\frac{1}{2} \times 7\frac{1}{4}$  inches. The former size is more likely to necessitate a cutting of the specimen but is more conservative of filing space than the latter size.

In the herbaria where the packets are pinned or pasted to sheets of heavy paper, boxes of various sizes are usually used in which to store the large, bulky specimens. The vertical filing system is also adapted to the filing of most of the larger specimens in this manner without their having to be cut. The box has a cover and its size is the same as that of the packet or envelope, except in thickness, and this is governed by the thickness of the specimen to be filed. A specimen in such a box may be filed in its proper order among the packets, thus avoiding segregation.

Where the packet is filed vertically without placing it in an envelope, the label is removed from the original packet and pasted to the standard packet, or a new label is made and the old one enclosed. In some cases, instead of making a new label, the data are typed or written on the upper fold or on the main body of the packet. If typed, the typing is done before the sheet of paper is folded into packet form. Where the packets are put into envelopes the original packet and its label are kept intact, or, if a new packet is made, the label is removed from the old packet and attached to or placed within the new one. If the latter plan is followed the data from the original label are written or typed on the outside of the envelope. In case the specimen is large enough to require a box which is to be filed vertically, either the original label or a new one is pasted on the outside of the box. If a new label is made the old one is placed in the box with the specimen.

The vertical system of filing specimens is especially well adapted to cross reference by means of a card index. The speci-

mens may be filed and indexed according to sequence of accession, according to host or according to order, genera and species. If the specimens are filed according to order of accession at least two card indexes are used, one listing the accessions serially and the other arranged according to host. A third index arranged according to genera and species is sometimes used. If they are filed according to order of accession a card similar to the one shown in figure 1 is used, and is usually filled out in writing. The data for the other one or two index files are obtained from this card and are usually typed. If the specimens are filed according to genera and species then only one card index system is necessary, namely, the host index, but in some cases a card index to fungus genera and species is also kept.

The use of the accession card as shown in figure 1 is probably self-explanatory. Heading the column on the left is the serial accession number. The genus and species of the fungus and the name of the author are placed on the top line. The remainder of the data are filled in as indicated. By checking or under-scoring one or more words in the left margin one may indicate location, method or methods of preservation, etc., of a given specimen. The particular marginal words shown in figure 1 may be changed or substitutions may be made in order to fit them to the needs of any given herbarium. If the arrangement in the herbarium is such that an accession card is not used then a similar marginal checking system may be used to advantage on the host or genera and species index cards. It is perhaps unnecessary to figure or enumerate the other data that should be placed on these cards, except to say that on the host index card sufficient space should be left for the recording of the herbarium numbers of a given parasite, especially if the specimens are arranged according to host.

In the herbarium of the New York State Museum such specimens as those of the Thelephoraceae, *Peridermium* and the lower fungi are placed in packets and the individual packets are filed vertically. At Cornell the Plant Pathology Herbarium and the specimens in Durand's collection are filed in the same manner. The same system is used also in the Bartholomew Herbarium at Hays, Kansas, at the University of Missouri,

Massachusetts Agricultural College, Oregon Agricultural College, and in the Plant Pathology Herbarium of Washington State College. In some of these herbaria all specimens have been reduced, where reduction was necessary, to such a size that they could be placed in standard-size paper packets or in cardboard boxes which are filed vertically in their proper places among the packets.

**ADVANTAGES OF METHOD.**—It is conservative of both floor and filing space and a maximum number of specimens are available without the use of a step-ladder. Perhaps as many specimens per cubic foot of space could be filed in the herbarium where the packets are attached to large sheets and filed flat as where the packets are filed vertically, provided no vacant spaces were left on the large sheets in each filing compartment, but this is seldom if ever the case. As mentioned above this method is well adapted to the use of a card index system for cross reference. The easy accessibility of any single individual specimen is another favorable feature, except where the specimens have been filed serially in order of accession. By this method the specimens can be more easily rearranged according to a different classification, if such a change should appear to be desirable. Drawings, lantern slides, photographs and permanent microscopic mounts on glass slides may be filed in or adjacent to the packet or envelope containing the specimen. Other features which make this method of filing desirable are that it is necessary to handle only one specimen at a time, danger of breakage through handling is reduced to a minimum, the specimens are probably less accessible to insects and are as nearly as possible free from dust and smoke fumes, especially if the packets are placed in envelopes.

**DISADVANTAGES.**—Probably the greatest objection to this method of filing is that it may require the cutting of either a host or a fungus in order to get it into the packet, envelope or box. However, if the packets are at least  $4 \times 6$  inches in size and if the boxes of similar size are used in conjunction with these, most of the specimens may be filed without having to resort to an objectionable amount of reduction. Where specimens are filed in this manner a mycological systematist would probably find it more cumbersome than by the method described

under the first heading. This would be especially true if each packet were filed in an envelope. For example, if several specimens of a given species were to be examined at the same time each packet would have to be removed from its envelope before the packet could be opened, while if the packets were attached to herbarium sheets the opening of the folder would at once expose all of the packets containing the specimens of a given species. While the filing of specimens in the order of accession is conservative of space and may have other advantages, yet such a system would be very inefficient for the use of a mycological systematist. One herbarium drawer might not only contain many species but also many genera; specimen number 500 might be an *Aecidium* and 501 a *Sphaerotheca*. If the packets are not placed in envelopes there is some danger that the end flaps may become doubled-over at the corners or may make more or less trouble in filing. By filing the packets vertically the specimens not only tend to shift to the bottom of the packet, but there is also danger that by overcrowding some of the more fragile specimens may be injured. Furthermore, this method, though using a minimum amount of floor and filing space, calls for a greater expenditure of time and labor, and for actual filing material such as paper for the making of standard-size packets for envelopes, for guide cards, and for the cards to be used for indexing.

### 3. Specimen Packets on Sheets Bound in Book Form

Comparatively few mycological herbaria in the United States have specimens filed in book form. Harvard, the University of Illinois, and the Connecticut Agricultural College may be cited as having at least a portion of their mycological specimens filed in this manner. In most herbaria the packets are removed from such volumes and filed in the same manner as the other specimens. This method, however, has the advantage that collections of exsiccati, for example, may thus be kept intact, preserving the individuality of the persons issuing them and also the nomenclature of the periods in which they were issued.

This method has the disadvantage that it is expensive, a special type of filing case is required, and the specimens of a given

species cannot be easily segregated. The last objection may be partially overcome or compensated for by using a loose-leaf volume, or by numbering the pages and making a card index reference list of the specimens.

#### 4. Specimens Filed in Boxes

Large, bulky specimens, such as certain of the Basidiomycetes, are not usually filed in packets but in cardboard boxes of suitable size. The very small, fragile specimens of this same group or of other groups are sometimes filed in very small boxes and these

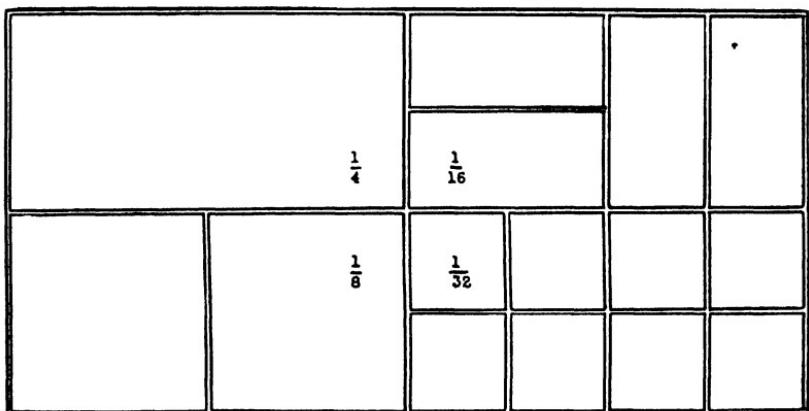


FIG. 2. An illustration of how small boxes used for bulky specimens may be nested in the large herbarium boxes, the small boxes being  $\frac{1}{4}$ ,  $\frac{1}{8}$ ,  $\frac{1}{16}$ , and  $\frac{1}{32}$  the size of the large ones.

in turn are glued to the inside of the packet. To keep these very small specimens from being injured by rattling around in the box one or more of the following methods may be used. The interior of the box may be padded with cotton, or some prefer very soft cloth or tissue paper, thus avoiding any annoyance from loose cotton fibers; the specimen may be glued to the interior of either box or lid, the latter permitting more satisfactory inspection; it may be pinned to either box or lid by using short pins on the inside or longer ones which enter from the outside; to facilitate ease of specimen inspection it may be pinned or glued to a card which fits snugly into the box but which is not permanently attached to it; a slim-pointed thumb tack with its head glued to the bottom of the box may be used upon which to impale certain kinds of these specimens.

In a herbarium where the packets are attached to sheets, two groups of boxes (usually cardboard) are generally used in which to file the bulky specimens. The first group is composed of small boxes of various size, into which the specimens are placed, and the second group is composed of large boxes which serve as containers for the small ones. The large boxes have covers; the smaller ones may have covers, but usually do not. The lateral dimensions of the large box are usually such that it will just fit into the regular filing compartment, while its depth is usually such that from one to three may be filed, one on top of another, in the same compartment. For example, if the filing compartment is 5 inches high, one may be approximately  $2\frac{3}{8}$  inches deep and the other two approximately  $1\frac{1}{4}$  inches deep. The small boxes vary in size but are such that they can be nested within the large boxes without loss of space. Figure 2 illustrates how different combinations of the smaller boxes may be filed within the larger ones. Within the large box any two of the smaller boxes may be replaced by the one that is twice as large, or conversely, the larger sizes may be replaced by two that are just half as large, four that are one-fourth as large, etc. For the sake of neatness, the small boxes in a given large box should be of the same depth, and that depth should be just a little less than that of the large box in which they are filed. However, in some herbaria which have been examined the small boxes vary in depth according to the size of the specimen which they contain. Some herbaria also use only two sizes of small boxes,  $2 \times 3$  and  $4 \times 6$  inches, with depths ranging from  $\frac{1}{2}$  inch to 2 inches. In another herbarium the small boxes (covered) are of six different sizes, ranging from  $3 \times 2 \times \frac{5}{8}$  to  $6 \times 8 \times 2\frac{1}{2}$  inches. These are placed in large covered cardboard boxes, approximately  $16\frac{1}{2} \times 18\frac{1}{2} \times 4$  inches, two of which are filed side by side in a herbarium compartment.

In at least one herbarium the Clavariaceae and most of the resupinate Thelephoraceae and Hydnaceae are not put into the small boxes, but are put into packets ( $5\frac{1}{2} \times 3\frac{5}{8}$  inches) made of stiff paper and filed vertically in the large boxes along with the smaller boxes, which preserves the natural order, or in covered boxes of full length and height, but only  $5\frac{3}{4}$  inches wide.

In some cases covered cardboard boxes of suitable size are used for filing the forms that cannot be readily placed in the packets, but instead of nesting these small boxes within larger ones they are glued to stiff cardboards of the same size as the regular herbarium sheet. These are then pigeon-holed in proper order along with the genus and species folders which contain the flat specimens.

One herbarium, economizing on box expense, does not use the large box of herbarium-sheet size, but uses shoe boxes into which smaller boxes are nested. These boxes are about  $6 \times 12$  to  $12\frac{1}{2} \times 3\frac{3}{4}$  to 4 inches in size. They can be had in almost any quantity merely for the asking and can be discarded when they become broken or soiled. This method should be regarded as only temporary.

Under this heading may be mentioned again the fact that in several herbaria where the vertical system of filing is used the boxes in which the larger specimens are to be placed are of the same lateral dimensions as the packets and are filed vertically in their proper sequence among them. The thickness of these boxes usually ranges from  $\frac{1}{2}$  to  $2\frac{1}{2}$  inches.

Where the smaller boxes are filed within the larger ones the genus is indicated on the end of the large box. The species labels are either placed loosely in the small boxes or are attached to them. The latter plan is usually followed if the specimens are subject to more or less constant use.

##### 5. *Other Methods of Filing Specimens*

The methods already described are the ones principally used in the strictly mycological herbaria. However, in some of these and more especially in pathological herbaria some or all of the following additional methods are used. For purposes of demonstration certain specimens may be preserved in liquid, such as alcohol or formalin, in a glass container of appropriate size. Such specimens may be previously treated in such a manner as to preserve their natural colors. Other specimens suitable for demonstration may be enclosed in a Riker or similar mount; in special stationary cases of glass, or at least with glass doors; in shallow hinged cases which may be either open or fitted with

glass doors; still others in balsam, or some other suitable medium, on microscopic slides.

#### FILING CASES

It is not the intention to discuss in this paper the different types of filing cases that may be used in which to keep mycological specimens. However, it is thought that one or two illustrations may be of interest. Figure 4 shows a view of the herbarium cases and work tables in the Farlow Cryptogamic Herbarium at Harvard.<sup>6</sup> Plate 22 shows large metal cases on the main floor and in two galleries of the Gray Phanerogamic Herbarium at Harvard.<sup>7</sup> Although the Gray Herbarium is phanerogamic instead of cryptogamic the conservative use of space and the arrangement of the filing cases is of sufficient interest to make it worth while to include this illustration. These cases are arranged for the filing of the standard-size herbarium sheets in folders and would therefore be suitable for the filing of mycological specimens on similar sheets. At A and B on the gallery railings may be seen glass shelves upon which the folders are laid while specimens are being examined. Note also the desk or counter type of metal case, several of which are placed on the main floor. These cases as in the Farlow Herbarium are made as nearly as possible dust and insect proof; therefore they may be readily fumigated if such becomes necessary.

#### DISCUSSION

While some bulky mycological specimens are preserved in liquids they are usually either dried so that they will as nearly as possible retain their natural form, or they are cut or pressed so that they may be placed in a smaller container than if preserved whole. The filing of the whole specimen entails less work than the latter method, but it requires considerably more filing space. The objection is often raised that the cutting or pressing of the specimens destroys their natural size and form. However, if care is taken this can be very largely avoided if boxes no smaller

<sup>6</sup> The photograph for figure 4 was kindly furnished by Dr. Wm. H. Weston of the Laboratories of Cryptogamic Botany of Harvard University.

<sup>7</sup> The photograph for plate 22 was kindly furnished by Dr. H. M. Fitzpatrick of Cornell University, through the courtesy of the Art Metal Construction Company of Jamestown, N. Y., which made the cases.

than  $4 \times 6$  inches and of appropriate thickness are used as containers for such specimens. By using a box of this size and by using discretion in cutting, a sufficient number of matched pieces can be preserved so that the form of the specimen may be retained. It is a rare case that the specimen is too large to be at least fairly satisfactorily preserved in this manner. Furthermore, the cutting of the specimen in this manner is considered by many to be an advantage rather than a disadvantage. For example, the cutting of a *Peridermium* specimen exposes the macroscopic effect of the parasite upon its host; the cutting of an *Agaricus* discloses the lateral surface of the gills and the gross structure of the pileus; and the cutting of a *Polyporus* permits of a lateral view of the pores.

Pressing a thick, bulky specimen is usually much more objectionable than cutting because of the resulting distortion. In the case of fungi on leaves or stems it would seem that there can be no real serious objection to the necessary reduction of the size of the larger specimens to the size of the standard container, provided the container is of such a size that it will meet the average requirements of minimum cutting and maximum conservation of filing space. It seems only reasonable that this should be an important governing factor in the filing of all specimens, whether flat or bulky, and whether filed flat on sheets or in boxes, vertically on half-sized sheets, or vertically in packets and boxes. Sooner or later every actively growing herbarium will demand a reduction in amount of filing space per individual specimen or the enlargement of the herbarium as a whole.

The neat and accurate folding of a sheet of paper into the form of a packet requires considerable skill and practice. The use of a pattern made of a thin but stiff paper card has been found to be very helpful. A piece of sheet aluminum of only sufficient thickness to give the required stiffness has also proved very satisfactory. The card or metal of the desired size is placed on the sheet of paper and the folds made accordingly. The pattern can be removed quickly by opening one end of the packet. This is especially true if a metal pattern is used; the weight of the metal will cause it to slide very easily and quickly from the

open end of the packet. These packets are later placed in a letter press or under a weight where the creases are made permanent. Many packets are made without the use of a pattern and thus probably a little more rapidly, but the time gained is

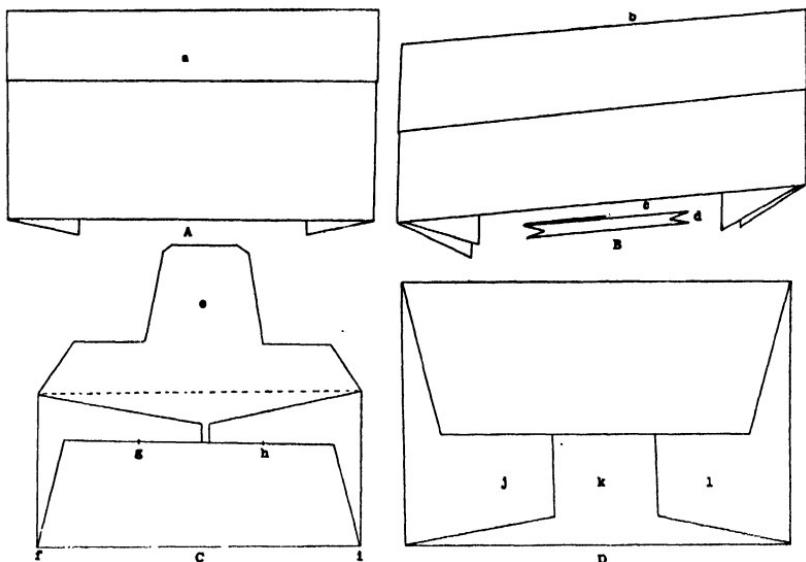


FIG. 3. Some of the different methods of folding packets which are used in mycological and pathological herbaria.

usually at the expense of neatness and accurateness. The flap which is folded over from the top and which is to bear the legend should preferably reach almost to the bottom of the packet. While this uses more paper it lends stiffness to the packet, thus protecting its contents, and also helps to prevent excessive bulging and gaping. Such a packet is also more easily put into the vertical file. If the packet is to be filed vertically the end flaps should be at least  $1\frac{1}{4}$  inches long to facilitate filing and to prevent bulging, but if it is to be pasted or pinned to a herbarium sheet the ends should not exceed  $\frac{3}{4}$  of an inch in length or there would be possible danger of injuring the specimen when opening or closing the packet.

Figure 3 illustrates the appearance and methods of folding four different types of packets. *A* is the type that is probably most commonly used in the United States. The length of the flaps at

the ends and the width of the marginal fold at *a* may be varied to suit individual needs. *B* is made on the bellows plan at the upper and lower edges. Such a packet is considerably more difficult to make, but is considered to be more flexible, to give more room for the specimen and also to give it more protection, being thicker at the edges. The drawing *d* represents a cross-section of *B* at *c b*. *C* represents a type of packet received from the Kew Garden herbarium. The flaps from *f* to *g* and *h* to *i* are sealed. The flap *e* is inserted under the portion *g* to *h* which is left unsealed. While there would be little danger of losing any portion of a specimen from such a packet it would be more difficult to insert or remove it. *D* is not an uncommon type of packet. None of the flaps are sealed and often are much shorter than indicated here. The flap *k* may be placed outside of *j* and *l* rather than as indicated in the drawing. Such packets have the advantage that they may be machine cut and folded, but there is more danger of the packet bulging open, especially if the end flaps are short.

In some herbaria it is a common practice to glue the specimen to the packet, to the box or to a card which is placed in a packet or box. While some specimens are so fragile that they require special care to prevent their injury it would seem that in at least most cases padding or pinning would be preferable to gluing. One of the values of a herbarium specimen rests in its accessibility for examination. In some cases sectioning of a specimen is desired and this is often difficult or impossible if it has been glued to its receptacle.

It is not the purpose of this paper to discuss the different classifications that may be followed in arranging mycological material. It may be stated, however, that as various herbaria differ in their methods of filing they also differ as to methods of classification arrangement. For example, in one herbarium the arrangement may be according to North American Flora, while in another it may follow Engler and Prantl. In at least one herbarium the specimens are filed and numbered according to Saccardo's *Conspectus Systematicus Generalis*, and *Sylloge Fungorum*. As the specimens are given their number a check is also made opposite the species in the publication and future references to a given specimen are made through this channel.

Perhaps other methods than those mentioned in this paper are used for filing mycological specimens, or perhaps there are modifications of these methods. If so, it is only further proof of



FIG. 4. A view of the filing cases and work tables in a portion of the Farlow Herbarium at Harvard University. (Photo by Dr. Wm. H. Weston).

the lack of uniformity which exists and which makes it difficult for visiting systematists or plant pathologists, to say nothing of those who constantly use them, to make efficient use of the herbaria. Probably the principal reasons for this lack of uniformity are (1) that in most institutions only a very limited amount of money is available for the building up of a herbarium, (2) often those who have started the herbarium have had little or no experience in such matters, and those who later take up the work are equally inexperienced or for financial reasons are forced to follow the original method, and (3) by the time the experienced curator is obtained the task necessary for the desirable rearrangement and revision is so great that the time required for their accomplishment is generally prohibitive.

At the present time it cannot be said that there is a "standard" method of filing and classifying mycological specimens. Possibly

such a condition will never exist because there is considerable room for difference of opinion as to just what the standard method should be.

The endeavor here has been to outline, without too much detail, the present methods and possibilities of filing specimens in mycological herbaria. The attempt has been made to present the matter in as nearly as possible an unprejudiced manner, the feeling being that the opinion of one man in such a matter would have comparatively little value. It is hoped, however, that those who are particularly concerned in such matters may be stimulated to further thought and discussion, in order that a greater uniformity in herbarium methods ultimately may be established. In the meantime it seems feasible to suggest that before rearrangement is begun in an already existing herbarium or in beginning a new herbarium, a careful study of the different methods in vogue at the present time should be made. Every herbarium curator should have in mind that it is his task to file the greatest number of specimens in a given space in such a manner that they will be most readily available for study, most nearly in natural form, and least likely to be damaged by handling or otherwise.

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BERKELEY, CALIFORNIA.

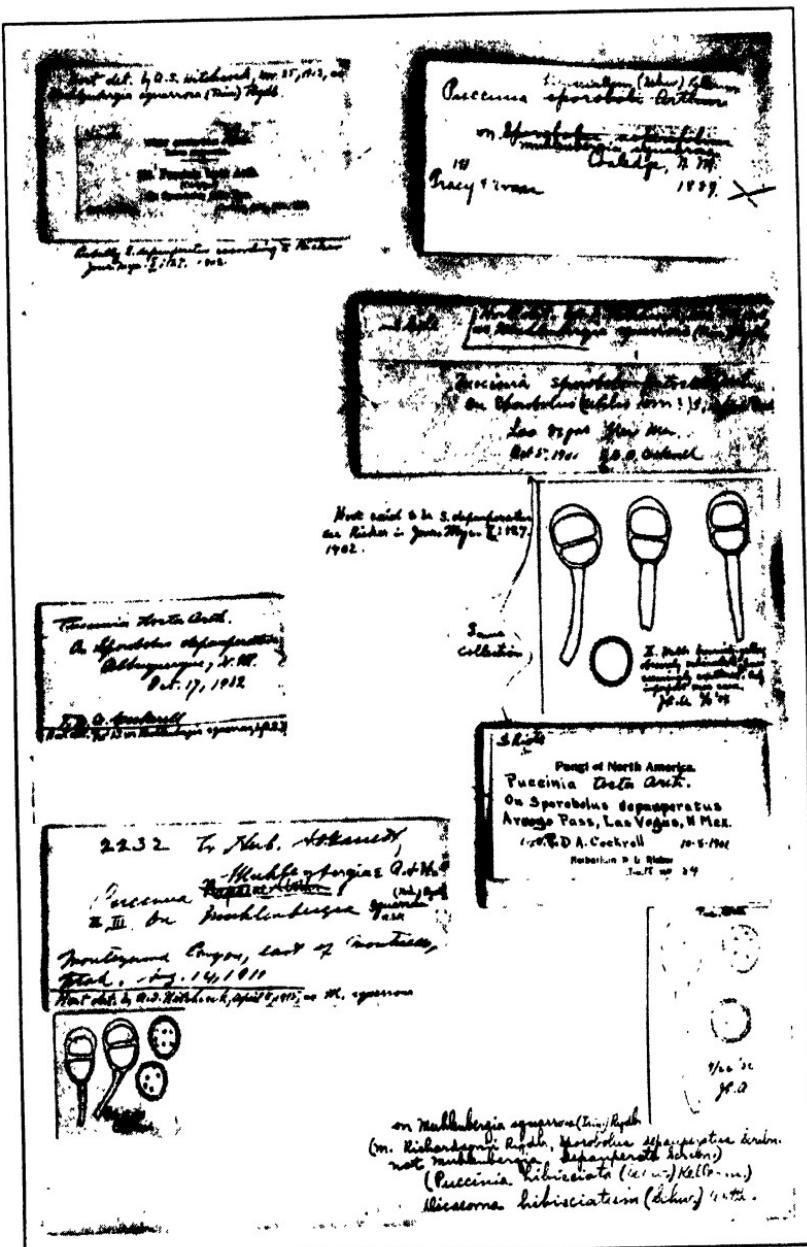
#### EXPLANATION OF PLATES

##### PLATE 21

Illustration of how packets, drawings, notes, etc., may be filed side by side on a single large herbarium sheet and thus be readily available for study at a moment's notice. (Photo by Dr. J. C. Arthur.)

##### PLATE 22

Type and arrangement of metal cases in the Gray Herbarium of Harvard University. Such cases are convenient and efficient for the filing of large herbarium sheets to which the small specimen packets are attached (Photo by Art Metal Construction Company).



SAMPLE SHEET FROM ARTHUR HERBARIUM





GRAY HERBARIUM, HARVARD UNIVERSITY



## NOTES AND BRIEF ARTICLES

The explanation of Figures 9 and 10, Plate 24, Volume 22 of *MYCOLOGIA* should read as follows: 9. Ascus of *Physalospora Zeae* containing eight spores; 10. Ascus of *Physalospora Zeae* containing four perfectly formed spores.

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Mr. Robert Hagelstein, Honorary Curator of Myxomycetes, in The New York Botanical Garden, sailed February 11th for a three weeks collecting trip in Trinidad and Barbados, his time having been chiefly devoted to Myxomycetes and Diatoms.

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✓ To correct a confusing error, the publishers, McGraw-Hill Book Company, Inc., 370 Seventh Avenue, New York, N. Y., have recently reprinted page 198 of my book, *The Lower Fungi-Phycomycetes*. A copy of this page will be mailed free by the publisher, or by the author, to every owner of the book who writes requesting it.—H. M. FITZPATRICK.

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A Memoir of the Torrey Botanical Club (Volume 18, No. 1) recently issued consists of 108 page discussion by Professor Herbert S. Jackson of Toronto University on "Present Evolutionary Tendencies and the Origin of Life Cycles in the Uredinales." The paper was presented in part before the Mycological Section of the International Botanical Congress, held at Ithaca, New York, August, 1926.

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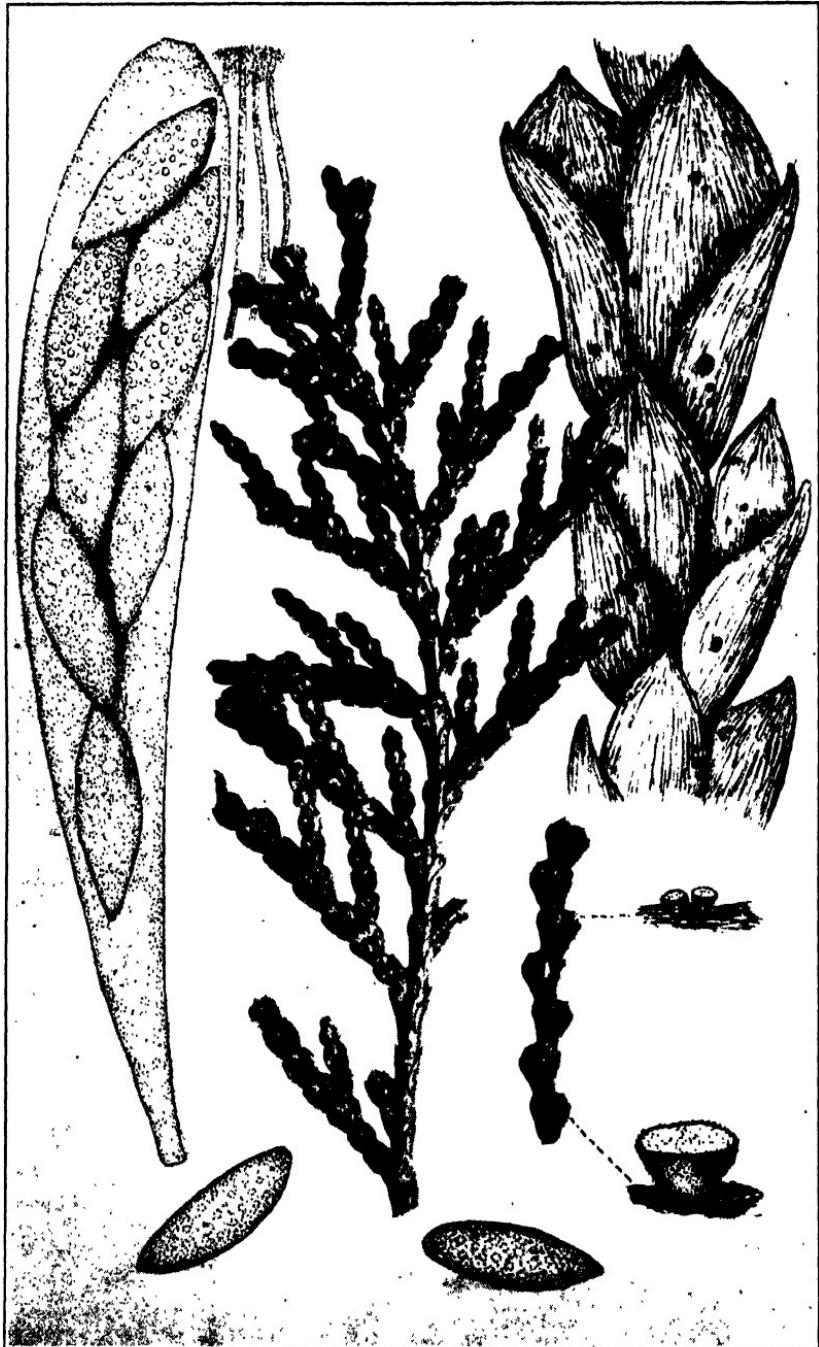
The January issue of the American Journal of Botany contains an article by Ernst J. Schreiner on "Two species of *Valsa* causing disease in *Populus*." The two species reported as responsible for cankers in poplars are *Valsa sordida* Nitschke, the pycnidial stage of which is *Cytospora chrysosperma* (Pers.) Fries and *Valsa nivea* (Hoff.) Fries with a conidial stage *Cytospora nivea* (Hoff.) Sacc. The disease caused by these two species of fungi are quite

similar. A great deal of experimental work has been conducted by Dr. Schreiner both in the greenhouse and in the field.

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"Mycological Explorations of Colombia" by Carlos E. Chardon and Rafael A. Toro was issued in the Journal of the Department of Agriculture of Porto Rico 15: 195-369, *pl. 30-35*. 1930. Several mycologists have collaborated in this work the Myxomycetes being treated by W. C. Muenscher; the Phycomycetes by W. H. Weston; the Pezizales by F. J. Seaver; the Xylariaceae by J. H. Miller; the Cercosporae by C. Chupp; the Ustilaginales by H. S. Jackson; the Uredinales by F. D. Kern and H. H. Whetzel and the Eubasidiomycetes by L. O. Overholts. A host index is appended. This is a very presentable contribution to our knowledge of South American fungi and includes the descriptions of many new species.





*CHLOROSCYPHA SEAVERI*

# MYCOLOGIA

VOL. XXIII JULY-AUGUST, 1931

No. 4

## PHOTOGRAPHS AND DESCRIPTIONS OF CUP-FUNGI—XIV<sup>1</sup>

### A NEW GENUS

FRED J. SEAVER

(WITH PLATES 23 AND 24)

In November, 1911, the writer sent to Dr. H. Rehm of Germany among other things a cup-fungus on the foliage of white cedar from Montana. On January 7, 1912, Rehm reported on this species which he called *Helotium Seaveri* Rehm, n. sp. Although Rehm lived until April 1, 1916, and continued to publish up to 1914, the writer has been unable to find any record of the publication of this species, although it is possible that it may have been published by him. The material was filed away in the collection until recently when it became subject to rather critical study.

In working over the inoperculate cup-fungi, preparatory to a monograph, the writer has encountered a second species, on leaves of *Sequoia* from California, with characters very similar to those of our own. The form on *Sequoia* was described by Phillips and Harkness as *Peziza chloromela*. Saccardo later transferred this to *Chlorosplenium* because of the greenish color of the apothecia. This and the preceding are without question congeneric although they can scarcely be regarded as belonging to the genus *Chlorosplenium*.

<sup>1</sup> This paper is preliminary to a monograph of North American Cup-fungi (inoperculates), a companion volume to North American Cup-fungi (operculates) which was published by the author and issued in December, 1928.

[*MYCOLOGIA* for May-June (23: 159-246) was issued May 1, 1931]

On further investigation a third species was found in the collection described by Ellis as *Dermatea juniperina* on leaves of *Juniperus communis* from Iowa. This again appears to be congeneric with the other two although the spores are somewhat different in form.

In December, 1930, the writer received from Dr. H. S. Jackson of Toronto, Canada, a fourth species on *Thuja occidentalis* which is very similar to our own form on *Thuja* but with spores which are much more slender and apothecia which are stipitate instead of sessile or subsessile. All of the four species occur on the foliage of coniferous trees and all are apparently parasitic. Since they have much in common and do not fit well in any of the known genera the writer proposes to establish a new genus for these forms. It seems very likely that other forms will be brought to light on further investigation. The name *Chloroscypha* is suggested by the fact that the apothecia are decidedly yellowish-green by transmitted light. They belong to the inoperculate section of the cup-fungi.

### CHLOROSCYPHA Seaver, gen. nov.

Apothecia gregarious or scattered, sessile or stipitate, minute or of medium size, yellowish-green to blackish when dry, the substance yellowish-green by transmitted light and resembling that of *Ascobolus*, occurring on the foliage of conifers, *Thuja*, *Sequoia*, and *Juniperus*, and apparently parasitic; asci when young greenish, normally 8-spored; spores comparatively large, at first granular and appearing greenish, but hyaline when mature, typically fusiform or more rarely broad-ellipsoid; paraphyses slender, simple or branched, surrounded by a greenish matrix.

Type species, *Helotium Seaveri* Rehm.

Occurring on foliage of *Thuja*.

Apothecia subsessile; spores broad-fusoid.

1. *C. Seaveri*.

Apothecia stipitate; spores narrow-fusoid.

2. *C. Jacksoni*.

Not on *Thuja*.

On *Sequoia*; spores fusoid.

3. *C. chloromela*.

On *Juniperus*; spores broad-ellipsoid.

4. *C. juniperina*.

**1. *Chloroscypha Seaveri* (Rehm) Seaver, comb. nov.**

*Helotium Seaveri* Rehm (in litt.). 1912.

Apothecia minute, scarcely exceeding .5 mm. in diameter, subsessile, occurring singly or in small cespitose clusters on the leaves of the host, turbinate, greenish, becoming almost black in dried material; hymenium plane or nearly so, lighter than the outside of the apothecium, the substance of the apothecium pale olivaceous-green when teased out and viewed by transmitted light; asci clavate, reaching a length of 100–135  $\mu$  and a diameter of 25–30  $\mu$ , the contents greenish, 8-spored; spores irregularly 2–3-seriate, fusoid to fusiform, the lower end often more pointed than the upper, densely filled with granules and slightly yellowish-green, about 8–9  $\times$  25–28  $\mu$ , smooth or very minutely roughened; paraphyses filiform, scarcely enlarged above.

On foliage of white cedar, *Thuja plicata*.

Type locality: Libby, Montana.

Distribution: Known only from the type locality.

According to J. R. Weir, this fungus causes a very destructive blight. This species appears to be very closely related to the previously described *Chlorosplenium chloromelum* which occurs on the foliage of *Sequoia sempervirens* in California.

**2. *Chloroscypha Jacksoni* Seaver, sp. nov.**

Apothecia scattered, stipitate, at first closed, gradually opening and becoming shallow cup-shaped, then discoid, externally yellowish furfuraceous, becoming darker with age, reaching a diameter of 2 mm.; hymenium concave, plane or slightly convex, yellowish with a greenish tint, often becoming nearly black with age; stem slender, reaching a length of 2 mm., similar in color to the outside of the apothecium; asci clavate, reaching a length of 100–110  $\mu$  (rarely 130) and a diameter of 12–14  $\mu$ , 8-spored; spores irregularly 2-seriate, fusoid or fusiform, often with two distinct oil-drops or granular, often apparently greenish when young, usually hyaline when mature, and often minutely roughened or smooth, 6–7  $\times$  20–28  $\mu$ ; paraphyses slender, rather abruptly enlarged above and surrounded with a greenish-yellow substance.

On *Thuja occidentalis*.

Type locality: Temagami Region, Canada (Jackson, 2047).

Distribution: Known only from the type locality.

This species differs from the preceding, which also occurs on *Thuja*, in the much narrower spores and stipitate apothecia. Whether this species is also parasitic has not been determined.

**3. *Chloroscypha chloromela* (Phill. & Hark.) Seaver, comb. nov.**

*Peziza chloromela* Phill. & Hark. Grevillea 13: 22. 1884.

*Chlorosplenium chloromelum* Sacc. Syll. Fung. 8: 319. 1889.

Apothecia scattered or gregarious, short-stipitate, externally smooth, greenish-black, reaching a diameter of .6 mm.; hymenium becoming nearly plane, yellowish-green; stem reaching a length of 1 mm., a little paler than the outside of the apothecium; asci clavate cylindric; spores clavate or fusiform, usually curved, at first hyaline, becoming greenish,  $4\text{--}5 \times 20\text{--}25 \mu$ ; paraphyses filiform, indistinct, adhering together.

On leaves of *Sequoia sempervirens*.

Type locality: California.

Distribution: Known only from the type locality.

A note from the Royal Botanic Gardens states that the material of *Peziza chloromela* at Kew is very scanty. Through the kindness of the Director of that institution a microscopic slide has been examined. The spores as indicated in the description are smaller than in the species on white cedar.

**4. *Chloroscypha juniperina* (Ellis) Seaver, comb. nov.**

*Dermatea juniperina* Ellis, Am. Nat. 17: 192. 1883.

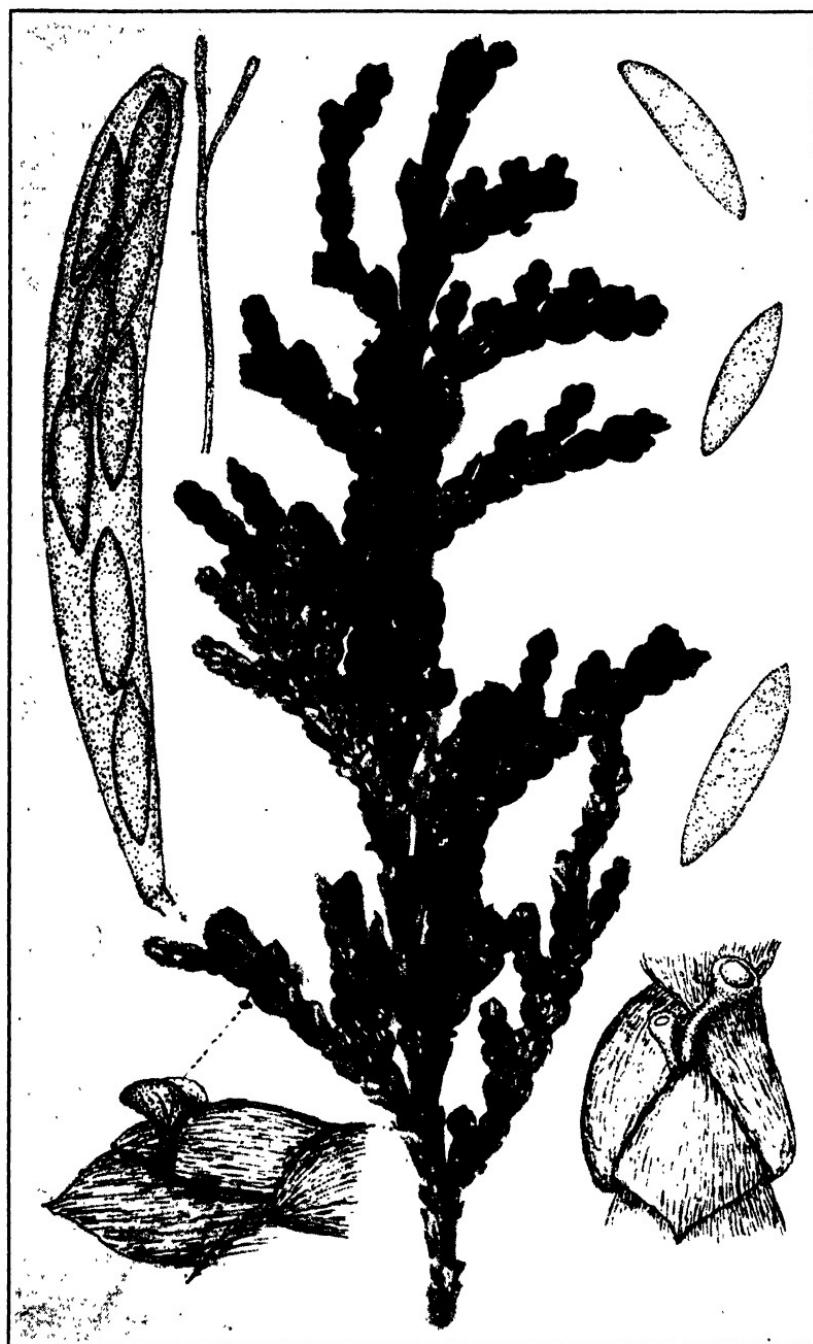
Apothecia gregarious, at first rounded, expanding and becoming turbinate, tapering into a stem-like base, black to the unaided eye, greenish with transmitted light, reaching a diameter of .25 mm.; asci clavate, reaching a length of  $130 \mu$  and a diameter of  $20 \mu$ , tapering rather abruptly below; spores ellipsoid or fusoid, about  $9\text{--}10 \times 18\text{--}20 \mu$ , granular within, often appearing greenish from the greenish material which surrounds the asci and paraphyses; paraphyses slender, enlarged above, reaching a diameter of  $4 \mu$ , adhering together at their tips, yellowish-green.

On leaves of *Juniperus communis*.

Type locality: Decorah, Iowa.

Distribution: Known only from the type locality.

This species is quite similar in general appearance to *Chloroscypha Seaveri* but differs in the form and size of the spores as well as in the host.



CHLOROSCYPHA JACKSONI



## EXPLANATION OF PLATES

## PLATE 23

*Chloroscypha Seaveri*. Center, photograph of foliage of white cedar, *Thuja plicata*, with fungus, slightly enlarged. Left, ascus with paraphyses and spores. Right, drawing of a piece of foliage showing distribution of apothecia much enlarged. Below, sketches of apothecia much enlarged and drawings of two ascospores.

## PLATE 24

*Chloroscypha Jacksoni*. Center, photograph of branch of *Thuja occidentalis* with fungus, about twice natural size. Left, drawing of ascus with spores and paraphysis. Right, drawing of several spores isolated. Below, sketches of portions of foliage with apothecia much enlarged.

Drawings of asci, spores and paraphyses were made with the aid of the camera lucida.

# TAXONOMIC STUDIES IN THE FAMILY PYTHIACEAE

## I. NEMATOSPORANGIUM<sup>1</sup>

C. P. SIDERIS

(WITH 12 TEXT FIGURES)

### INTRODUCTION

The studies presented herewith and in a series of papers to follow are the outcome of pathological investigations on a root-rot disease of pineapple plants known as pineapple wilt. As various microorganisms were isolated it became evident that many of them were undescribed members of the family Pythiaceae. Subsequent inoculations of roots of pineapple and certain other plants with these organisms proved that most of them are either weakly or aggressively pathogenic. In the attempt to identify them through the literature it was found that such literature was inadequate for the purpose.

This paper takes up first a review of the entire family Pythiaceae and points out the justification in the writer's opinion for reestablishing *Nematosporangium* as a genus. It then takes up specifically those of the fungi isolated that fall into this group, classifies them by means of morphological and cultural characters, and gives detailed descriptions of all new species. The technique on the isolation and pathogenicity of the different organisms is discussed in another paper, which will soon be published.

### FAMILY PYTHIACEAE

The family Pythiaceae, one of the three included in the order Peronosporales, is differentiated from the other two, namely, the Albuginaceae and the Peronosporaceae, by certain morphological features of the asexual reproduction of its members. The members of the family Pythiaceae lack a definite conidio-

<sup>1</sup> Technical paper No. 19 of the Experiment Station of the Association of Hawaiian Canners, University of Hawaii.

phore, whereas those of the other two families possess well defined conidiophores.

The family is divided by certain investigators (2, 9) into the genera *Pythium* and *Phytophthora*, and by others (11, 12) into the genera *Nematosporangium* and *Pythium*. The tendency on the part of the first group of mycologists is to incorporate the genus *Nematosporangium* in the genus *Pythium*, and on the part of the other, to place the genus *Phytophthora* in the family Peronosporaceae owing to the well differentiated conidia and conidiophores of some species of this genus.

The members of the genus *Nematosporangium* never produce conidia and those of *Pythium* possess only pseudo- or atypical conidia, that is, asexual reproductive organs that do not fall off the supporting hypha to grow vegetatively, but germinate while attached to the hypha. The members of the other two families possess euconidia or typical conidia. The genus *Phytophthora* occupies a very doubtful position, as some of its members produce both euconidia and pseudooconidia (chlamydospores). The pseudoconidia of spherical shape are produced more abundantly in old cultures and behave as resting spores and, in the majority of cases, remain attached to their supporting hypha. The euconidia of oval shape are produced in a greater degree in young and in a lesser degree in old cultures, and become mostly zoosporangia. They may or may not fall off their supporting hypha.

*Phytophthora*, therefore, occupies a transitional position between the Pythiaceae, on the one hand, and the Peronosporaceae, on the other. It is on account of these and other morphological characters that it is placed by some in the former and by others in the latter family. The writer's critical study of the situation indicates that *Nematosporangium* merits generic recognition, owing to many basic morphological and physiological differences separating the members of this genus from those of *Pythium*.

Fischer (7) divided the genus *Pythium* into the subgenera *Aphragmium*, *Nematosporangium* and *Sphaerosporangium*. The prosporangia of the first two are nematoid or allantoid but never or very seldom spherical; whereas those of *Sphaerosporangium* are always spherical to lemon-shaped. The difference between *Aphragmium* and *Nematosporangium* is in the septation of the

exit tube of the prosporangium supporting the zoösporan-gium. This difference has been found later to be of small importance and too insignificant to merit subgeneric recognition. Butler (2), who studied Fischer's organism *Pythium complens* and Schenk's *P. gracile*, and de Bary's *P. reptans*, which Fischer used as type specimens for the creation of the subgenera *Aphragmum* and *Nematosporangium*, states the case as follows: "I have had what is certainly de Bary's *P. gracile*, as well as the species which I have identified with Schenk's organism, in culture for many months, and have never observed the sporangia to be cut off, though the accidental appearance of this, as described under *P. monospermum*, was not rare. Fischer (1892, p. 398) also agrees with Schenk that septa are absent." Schröter (12) on the basis of Fischer's later findings, that is, that there were no morphological differences separating the subgenera *Aphragmum* and *Nematosporangium*, incorporated these two subgenera under the name *Nematosporangium* and raised the subgenus *Nematosporangium* to generic rank. Butler (2), although aware of Schröter's classification, admitted Fischer's original classification and suggested the incorporation of the subgenera *Aphragmum* and *Nematosporangium* in the subgenus *Aphragmum*, on the basis that "the validity of the character on which the subgenus *Nematosporangium* was founded in *Pythium monospermum* by Fischer is doubtful and under the circumstances it seems unnecessary to separate this species from the *Aphragmia*." Lindau (11) retains the original classification of Schröter, recognizing the generic rank of *Nematosporangium*. Fitzpatrick (8) suggested, in 1923, the generic name *Nematosporangium* in preference to *Pythium* in the organism *Pythium aphanidermatum* (Edson) Fitz. Gauman (9) has adopted the original classification of Fischer as modified by Butler.

#### COMPARATIVE MORPHOLOGY OF NEMATOSPORANGIUM, PYTHIUM AND PHYTOPHTHORA

Butler (2) discusses with great thoroughness the morphological characters of a considerable number of species of the subgenera *Aphragmum*, *Nematosporangium*, and *Sphaerosporangium* of the genus *Pythium*. In the present discussion an attempt is being

made to present the differences between *Nematosporangium* (Schröter's version) and *Sphaerosporangium*.

The statements of the various investigators make it clear that the different members of *Nematosporangium* are distinguishable from those of *Pythium* by the absence of spherical or lemon-shaped prosporangia and conidia (FIG. 1, *a, b*). The nematoid or allantoid prosporangia of *Nematosporangium* often undergo elongation and assume vegetative growth which should not be confused with the true germination of conidia of members of the genus *Pythium*. In the matter of zoospore production *Nematosporangium* and *Pythium* are alike only in the development as an outgrowth from the prosporangium of a vesicle or zoosporangium proper, in which the zoospores are borne. Resemblances quite analogous to this may be found between the genera *Phytophthora*, *Albugo*, *Basidiophora*, *Sclerospora* and, in part, *Plasmopara*, where the stage of the germ sac (true zoosporangium or vesicle) has almost entirely been suppressed, that is, the mature zoospores swarm directly out of the prosporangium; yet there is no question as to generic differences between these organisms.

The mycelium in *Nematosporangium* is very irregular with outgrowths of different shapes and sizes, whereas that of the majority of members of the genus *Pythium* is fairly uniform in shape, although it may vary to an appreciable extent in size. There are, however, certain new species of *Pythium*<sup>1</sup> that exhibit a close resemblance to the *Nematosporangium* type of mycelium. The mycelium of species of *Pythium* has never been observed by the writer to form bud-like outgrowths or plasmatoögoses<sup>2</sup> in the tissues of hosts, whereas that of *Nematosporangium* always does.

The oögonia and oöspores of the members of both genera are always spherical, except for irregularities in some of the oöspores developing inside the tissues of hosts. The surface of the oöspore wall of all the species of *Nematosporangium* so far studied is smooth, except for slight wrinkles that may be found on the oögonial membrane of those species not filling the oögonium,

<sup>1</sup> Described in a paper following on *Pythium*.

<sup>2</sup> Plasmatoögosis(es) from the Greek πλάσμα and ὄγκωσις, plasmatic outgrowths or the bud like outgrowths of Butler (2).

whereas that of those of *Pythium* may be smooth, reticulate, or echinulate. The oöspore may or may not fill the oögonium in both *Pythium* and *Nematosporangium*. There is a considerable variation in the size of the oöspores of both genera under cultural and, to a considerable degree, under natural conditions. The great or small number of oöspores correlated with an early or late production inside the tissues of hosts is characteristic of many species of both genera and may be used for the identification of closely related species. The shape and size of the fertilization tube, its position in relation to the antheridium, and also the position of the antheridium in respect to the oögonium, the number of antheridia produced on the same hypha, and the number attached to an oögonium, constitute valuable characters for the identification of species.

The most outstanding differences between *Nematosporangium*, *Pythium*, and *Phytophthora* are in their zoöspore-producing organs. Those of *Nematosporangium* and *Pythium* are divided into three morphologically different parts, prosporangium, exit tube, and zoösporangium. The prosporangium serves as reservoir of the protoplasm destined for the development of zoöspores. The exit tube forming simultaneously with or slightly before the development of the zoösporangium separates the prosporangium from the zoösporangium and serves for the passage of the protoplasm from the former to the latter organ. The wall of the prosporangium is a continuation of the exterior wall of the hypha supporting this organ, whereas that of the zoösporangium is not, but constitutes a part of the so-called ectoplast of the prosporangium. The zoösporangium is of short duration; it emerges from the emission collar almost simultaneously with the flowing protoplasmic contents of the prosporangium and lasts until the zoöspores are completely formed and have escaped into the surrounding medium. The corresponding organs in *Phytophthora* vary widely from this. The three genera may be differentiated as follows:

*Nematosporangium*: Prosporangia (being morphologically identical with plasmatoögoses) are not well defined structures. They may be nematoid, allantoid, or rarely subspherical, intra- or extra-marginal; exit tube very long; zoösporangia spherical,

size variable; zoospores few to many; conidia unknown (FIG. 1, *a*, *b* and *c*).

*Pythium*: Prosporangia well defined, pithoid,<sup>3</sup> spherical to ovoid, mostly extramatrical, rarely intramatrical; exit tube short; zoosporangia spherical, size variable; zoospores few to many; pseudoconidia present in certain species (FIG. 1, *d* and *e*).

*Phytophthora*: Prosporangia well defined, not pithoid, lemon-shaped to spherical but without a prominent neck, mostly extramatrical of different sizes; zoosporangia developing within the walls of prosporangia; exit tube entirely lacking or rarely slightly developed; zoospores few to many, pseudo- and euconidia present (FIG. 1, *f*, *g*, *h* and *i*).



FIG. 1. *a*, zoosporangium of *Nematosporangium* sp. and prosporangium in the cell of the host ( $\times 600$ ); *b*, drawing of a zoosporangium of *Nematosporangium* sp.; *c*, zoosporangium of *Nematosporangium* sp. ( $\times 300$ ); *d*, drawing of a prosporangium and zoosporangium of *Pythium* sp.; *e*, two prosporangia with their zoosporangia of *Pythium* sp.; *f*, drawing of the prosporangium-zoosporangium of *Phytophthora* sp.; *g*, prosporangium-zoosporangium with zoospores of *Phytophthora* sp.; *h*, prosporangium-zoosporangium discharging its zoospores ( $\times 300$ ); *i*, two emptied prosporangia-zoosporangia and an undeveloped one ( $\times 150$ ).

The separation of *Nematosporangium* from *Pythium* and the creation of two independent genera, by Schröter, was not only wise, but was also a necessity for a better understanding of the morphological characters and physiological behavior of the vari-

<sup>3</sup> *Pithoid* from the Greek *πιθώδης* = jug-like, that is, spherical to oval with a well defined open neck at the top.

ous members of the two genera. Fischer (7), Butler (2), and other investigators have not approved of the division of *Pythium* into two independent genera, because of a character common to both genera: *viz.*, the development of a vesicle, or zoösporangium proper, into which the zoospore or protoplasmic material is discharged by the prosporangium through a tube. It is rather unfortunate that the size, shape, and other characters of the prosporangium and exit tube in the two genera have been overlooked or characterized as unimportant.

Besides the morphological differences, there are physiological differences which point out that the members of the two genera stand out as two independent units. There is, doubtless, a certain relationship between *Nematosporangium* and *Pythium*, but not any greater than that existing between any two other genera of the same family.

#### THE GENUS NEMATOSPORANGIUM

This genus, then, as characterized above, is sharply differentiated from the other two genera of the family Pythiaceae. In differentiating species within the genus, one looks naturally first of all to morphological characteristics. These characters in closely related species, however, are not as prominent as cultural characters which have been used quite extensively for the differentiation of such species.

The morphology of the sexual organs constitutes a more reliable criterion for the differentiation of species than that of the asexual organs. The size of the asexual organs, namely, zoösporangia, depends on the size of the prosporangia. The latter organs vary considerably, as they may be composed of one or many lobes and thus contain either a small or a great volume of protoplasmic material which determines in turn the size of the zoösporangium and the number of zoospores.

The prosporangia of *Nematosporangium* being, as mentioned before, morphologically identical with plasmatoögoses can not be differentiated from them before they produce zoösporangia. They may consist of a single morphologically undifferentiated hypha of few to many microns in length (nematoid) or of morphologically differentiated hyphae of diameter many times that

of the unmodified or original hypha, with few or many lobes filled with protoplasmic matter (allantoid). They produce during the development of zoösporangia an exit tube or discharge tube, ranging in length between 25 and 300  $\mu$  or possibly longer, which, after opening at the tip, permits the outward passage of the vesicle and protoplasmic matter. The period required for the flow of the protoplasmic matter from the prosporangium to the vesicle or zoösporangium varies considerably, ranging between 10 and 40 seconds and depending mainly on the volume of such matter.

The zoösporangia of *Nematosporangium* vary considerably in size and in the number of zoöspores, the latter numbering from 4 to 50 or possibly more. In certain species, they form very readily in the culture media or in water, and in others, very slowly. In the latter case it was found necessary to take the dead roots of hosts that had been recently inoculated and killed by the organisms in question, place them in water and watch for the development of zoösporangia. The only species that have been observed to produce zoösporangia readily, when the aerial mycelium of the colony is used, are *Nematosporangium aphanidermatum* and *N. Butleri* (13). The others produce zoösporangia readily, only if the diseased tissue of the root of the host is used, as far as the author's observations have gone. The development of zoöspores within the zoösporangium requires from 15 to 20 minutes. The prosporangium first produces a discharge tube, which may measure from 20  $\mu$  to 300  $\mu$  in length. Then, at the tip of the tube appears the zoösporangium as a bubble-like sphere, first small, but increasing gradually until it reaches its maximum size. During the enlargement of the zoösporangium the protoplasm is continuously flowing in as a viscous mass. The latter process may last from few to many minutes and then starts the differentiation and development of zoöspores (FIGS. 4 to 12). The swarm stage lasts from 2 to 30 minutes and possibly longer, depending on the temperature of the environment. The zoöspores are reniform and biciliate in all the different species.

Plasmatoögoses are of great importance in the characterization of species. Plasmatoögoses may either group together and form

few large or many small tufts (intramatrical or extramatrical), or may be produced individually without the formation of aggregates. The size of such aggregates varies and is characteristic of certain varieties. Plasmatoögoses are also known by the name prosporangia. It is true that plasmatoögoses and prosporangia are morphologically identical, but until the latter produce zoösporangia one does not know definitely whether they are prosporangia or only protoplasmic accumulations that may produce a vegetative growth. Plasmatoögoses are analogous to the conidia of certain species of *Pythium* which may either become prosporangia if they produce zoösporangia or remain and germinate vegetatively and in the latter case be true conidia. The term prosporangia is inappropriate for these structures for the reason above stated.

On the basis of the morphology of the sexual reproductive organs, *Nematosporangium* may be divided into two sections, namely, *Polyandra* and *Oligandra*, the former including those members having many antheridia, usually 1 to 25, in relation to one oögonium, and the latter, those having few, usually one or two, in relation to one oögonium.

All of the members of the section *Polyandra* can be placed in three distinct groups or subsections on the basis of the time required for sexual reproduction, either in culture media or in the tissues of hosts. The members of the section *Oligandra* are placed in two subsections on the basis of their behavior in the development of zoösporangia. The subsections of the section *Polyandra* are as follows: (1) *Bradyspora*<sup>4</sup> including those members requiring very long time (5 to 25 days) and highly suitable culture media containing relatively high concentrations of nutrient substances; (2) *Hemibradyspora* those requiring a relatively short time (2 to 4 days) and highly suitable culture media; and (3) *Tachyspora*<sup>5</sup> those requiring a short time (1 to 3 days) in a great variety of culture media, even those containing relatively low concentrations of nutrient substances. The subsections of *Oligandra* are (1) *Plethorocomba*<sup>6</sup> and (2) *Oligocomba*.<sup>7</sup> The

<sup>4</sup> *Bradyspora* = slowly-produced spores, from the Greek *βραδύς* = slow.

<sup>5</sup> *Tachyspora* = rapidly-produced spores, from the Greek *τάχυς* = rapid.

<sup>6</sup> *Plethorocomba* = abundance of knots (referring to plasmatoögoses on the aerial hyphae), from the Greek *πληθώρη* = abundance + *κόμβος* = knot.

<sup>7</sup> *Oligocomba* = few knots; from the Greek *ὀλιγός* = few + *κόμβος* = knot.

former includes those members with many plasmatoögoses on their aerial hyphae, developing zoösporangia readily, and, the latter, those with very few plasmatoögoses developing zoösporangia rarely.



FIG. 2. *a, b, c, d, e, g, h, i, j, k, l*, oospores fertilized by single and compound antheridia, that is, antheridia produced by one per hypha or many per hypha produced on short branches arising from a common hypha; *f*, oögonium surrounded by a hypha previous to the production of antheridia; *m*, oospore showing the multi-branching of the antheridial hypha and the four antheridia produced on its branches.

The members of the subsection *Bradyspora* produce oospores rarely and few in number within the tissues of hosts; those of

the second subsection produce them occasionally, but in greater numbers in tissues containing a sufficient and suitable food supply and those of the third subsection produce them in the tissues of practically all the hosts so far studied. Members of the subsections *Bradyspora* and a few of *Hemibradyspora*, besides the long time they require for sexual reproduction, have their reproduction preceded by the formation of plasmatoögoses. These organs (FIG. 3, *a*, *b* and *c*), being filled with protoplasmic matter, serve possibly as storage organs in the subsections *Bradyspora* and *Hemibradyspora*, and thus make possible the wasteful process of sexual reproduction.

Plasmatoögoses of smaller sizes occur in the colonies of all species of the subsection *Tachyspora*. In this subsection, however, they either develop simultaneously with the oöspores or later, thus exercising no influence on the formation of oöspores. Such protoplasmic accumulations, called by many, prosporangia, may serve three purposes: (1) the development of zoösporangia, (2) development of oöspores and (3) continuation as storage hyphae, functioning as chlamydospores.

Some members of the subsection *Plethorocomba* produce oöspores very readily and mostly on the surface of the substrata and aërial hyphae and others slowly. For example, *N. aphani dermatum* produces oöspores very readily, whereas *N. Bulleri* produces them slowly. *N. Indigoferae* of the subsection *Oligocomba* produces oöspores extremely abundantly intra- and extramatrically and intra- and extra-cellularly. The oöspores of all the members of subsections *Plethorocomba* and *Oligocomba* are aplerotic.<sup>8</sup>

The antheridia in the various species of *Nematosporangium* vary considerably in size, shape and number per hypha (FIG. 2, *a*, *b* and *e*). The shape, in practically all of the species of *Polyandra*, is clavate and the length of the supporting stalk relatively long, although it varies considerably in certain species. The shape in the members of *Oligandra* is doliform and the length of the supporting stalk relatively short and in many cases almost lacking. The size of the antheridia of the different species varies

<sup>8</sup> Plerotic type = filling the oögonium, from the Greek πληρωτικός; aplerotic = not filling.

but slightly within the entire genus. The number of antheridia that may be borne laterally on a single hypha also varies. There are hyphae bearing many antheridia laterally which act as a single unit in the process of fertilization. Such hyphae usually encircle the oögonium and all of the antheridia become attached (FIG. 2, *d, f* and *g*). It is extremely difficult if not impossible to differentiate between the oögonial and antheridial hyphae pre-



FIG. 3. *a*, plasmatoögoses which may produce zoösporangia or serve as storage cells; *b*, *c*, plasmatoögoses: *b*, young, and *c*, old, the latter producing oögonia.

vious to the formation of such organs. The antheridia of *Nematosporangium* may be borne either on the same hypha bearing the oögonium or on separate hyphae. It seems that oögonia

influence the development of antheridia on nearby hyphae or on the same hypha either by endogenic or exogenic secretions or other such biochemical stimuli. That the reverse is not true is evidenced by the fact that the formation of oögonia always precedes that of antheridia.

The formation of oögonia in *Nematosporangium* depends on the quality and quantity of the available nutrients of culture media. Their size varies in the same proportion as oöspores. The number of antheridia, in relation to a single oögonium in the section *Oligandra*, including such species as *N. aphanidermatum* and *N. Butleri*, is limited to very few, 1 to 4, whereas in *Polyandra* it may vary from 1 to 12 and possibly more. Drechsler (5) reported as many as 25 in *Pythium arrhenomanes* Drechsler.

The oöspores of all species of *Nematosporangium* are morphologically alike, being smooth, spherical to subspherical, and surrounded by a wall varying in thickness between 1.2 and 2.0  $\mu$ .

Those of the section *Polyandra* are relatively large, averaging  $30 \pm 5 \mu$  in diameter in comparison to those of *Oligandra* which average  $20 \pm 5 \mu$ . The oöspores of all or most of the members of the section *Polyandra* are of the plerotic type whereas those of the *Oligandra* are of the aplerotic type. All of the members of *Oligandra* reproduce sexually very readily in comparison to those of the *Polyandra* (excepting *Tachyspora*). The oöspores of practically all species of *Polyandra* except some oöspores of *N. epiphanosporon* are produced in the substratum or on its surface, whereas those of *Oligandra* are mostly produced on the aerial mycelium.

#### NUTRITIONAL REQUIREMENTS OF NEMATOSPORANGIUM

The macroscopic or cultural characters of the organisms discussed in this paper have been observed to depend on the nutritional value and physicochemical properties of the various culture media. Appropriate culture media should be used as much as possible for the identification and standardization of these organisms. During these studies a very great number of culture media were tested and only those that favored the normal development, that is, the sexual and asexual reproductive processes of these various organisms, were selected.

The nutritional requirements of the various species of *Nematosporangium* for normal growth and sexual reproduction are exacting. There are very few food substances which possess properties for stimulating sexual reproduction in the various members of *Nematosporangium*. Sexual reproduction, being a very wasteful process, requires appreciable quantities of food substances that are rich in readily available nitrogenous compounds, fats, and sugars—substances from which protoplasm can be readily produced. Such substances as the seeds of plants and other parts of the fruit which are rich in such suitable food substances, as mentioned, make the best culture media for the growth of this group of organisms. Not all seeds but only those of a relatively small number of plants are suitable for this purpose. Chemical differences in the composition of the seeds of different plants are doubtless responsible for the suitability or nonsuitability of such seeds for culture media.

Synthetic culture media are not suitable for the sexual reproduction of some of these organisms.

#### CULTURE MEDIA

Various synthetic as well as natural culture media have been tested but only the latter gave good results. In spite of the variability within a species of the chemical composition of the pericarp of the fruit, there is little or no variability in the chemical composition of the seed. Seeds offer, therefore, the greatest degree of reliability for comparative studies of this type. For the preparation of the different culture media employed in these studies decoctions of the various vegetable substances were prepared. Such decoctions were then mixed with agar-agar for the preparation of solid media in the proportion of 1000 cc. of decoction to 17.5 grams of agar-agar.

The natural culture media used were as follows:

A. Cornmeal agar (*Zea Mays*). This was a preparation obtained from the Digestive Ferments Company.

B. Quaker Oats agar (*Avena sativa*). This was prepared by boiling 50 grams of Quaker Oats in 1000 cc. of water for 30 minutes. The mixture was filtered through cotton.

C. Flaxseed agar (*Cannabis sativa*). About 25 grams of

seeds are boiled for 30 minutes in 500 cc. of water and then strained through cotton. The residue is mixed once more with 500 cc. of water boiled for ten minutes and then strained. The first and second liquid portions are united.

D. Hemp seed and Quaker Oats agar. This is prepared by mixing 500 cc. of flaxseed decoction and 500 cc. of Quaker Oats decoction.

E. Cocoanut agar (*Cocos nucifera*). Only fresh, ripe cocoanut was used for the preparation of culture media. Two hundred grams of the endosperm together with the milk and 1000 cc. of water are hoiled for one hour and then filtered through cotton.

F. Melon-seed agar (*Cucumis Melo* var. honeydew). Twenty-five to 30 grams of melon seeds are ground up in a meat grinder, mixed with 1000 cc. of water and boiled for one hour. The mixture is filtered through cotton.

G. Watermelon-seed agar (*Cucumis Citrullus*). The preparation is identical with that of the melon-seed agar.

H. Pumpkin-seed agar (*Cucurbita Pepo*). The preparation is identical with that of the melon-seed agar.

I. Avocado agar (*Persea gratissima*). About 200 grams of the pericarp removed from the peel, mixed with 1000 cc. of water and boiled for two hours. The mixture is filtered through cotton.

J. Papaya agar (*Carica Papaya*). Only the ripe fruit of *Carica Papaya* is suitable for the preparation of media. One thousand grams of the peeled fruit is ground finely by means of a meat grinder, heated to boiling with 500 cc. of tap water for ten minutes, and then strained through cotton. The liquid is set aside and the solid residue mixed once more with 500 cc. of water, boiled for ten minutes and again strained. The first and second liquid portions are united.

K. Watermelon agar (*Cucumis Citrullus*). Both seeds and flesh of the watermelon were used for the preparation of this culture medium. The seeds were separated from the flesh and a decoction of them was prepared as described above under G. The juice extracted from a 6-pound watermelon by means of a fruit press (about one liter) was boiled for 20 minutes until the pigment coagulated and was then filtered through coarse filter

paper. The filtrate, a pale liquid, was mixed with an equal volume of the seed decoction.

*L.* Melon seed, flaxseed, and dextrose agar. Equal volumes of decoctions *C* and *F* are mixed. To 1000 cc. of the mixture 5 grams of dextrose is added.

A great variety of other substances have been used for the preparation of culture media in connection with these studies, such as potato (*Solanum tuberosum*), carrot (*Daucus Carota*), sweet potato (*Ipomoea Batatas*), bean sprouts (*Phaseolus Soja*) and combinations such as cornmeal, papaya, etc., but without any additional advantages.

The papaya culture media proved to be the best for reproduction and growth. It is recommended wherever the papaya fruit is available. The next best is the seeds of honeydew and watermelon. The pumpkin-seeds were not as satisfactory as the melon seeds. The chemical differences existing in these seeds, as given by Wehmer (15), cannot be used for ascertaining such properties as those required for the sexual reproduction of these organisms. The results obtained indicate that the nitrogenous and lipoid compounds must exist in a relatively high concentration in proportion to the carbohydrate substances in order to bring about normal sexual reproduction.

Besides the general requirements for sexual reproduction by the majority of species, there are certain species that refuse to reproduce except on few favorable media. This high specialization which is indicative of the influence of certain chemical compounds on the sexual reproduction of species of *Nematosporangium* emphasizes the importance of the substratum in the development of morphological characters and its bearing on the taxonomy of these organisms.

The behavior of these organisms in different culture media is analogous to that of bacteria. The development of morphological characters in the species of *Nematosporangium* may be compared with the biochemical changes resulting in the substrata of different species of bacteria. It is very difficult, if not impossible, to identify the various species of *Nematosporangium* on simply morphological differences of their various organs without using culture media that have been tested and proven to give variations in the growth and reproduction of these organisms.

## TECHNIQUE

The technique employed in studying the different organisms consisted in growing them on the various substrata mentioned, contained in Petri dishes. They were examined at 6- or 12-hour intervals by means of a compound microscope, using a 16 mm. objective and  $\times 15$  eyepiece, the Petri dishes placed on the stage closed and with their bottom sides up. By means of such precautions the contamination of cultures was reduced to minimum. Certain optical difficulties were met with in pigmented and turbid culture media. All these difficulties were finally overcome by using a very strong source of light (a 400-watt lamp). The magnification,  $\times 150$ , given by combining a 16 mm. objective with a  $\times 15$  eyepiece was found sufficient and satisfactory for this type of work.

It has been observed that all the different kinds of culture media, mentioned above, form some precipitate, consisting mostly of coagulated albumins or other proteins, during their sterilization in the autoclave. Such precipitates should neither be filtered out nor left at the bottom of the test tube when the contents of the latter are poured into Petri dishes, but should be shaken well with the clear liquid and poured together. They have been observed to be indispensable for the initial development of oögonia, especially with members of the subsection *Hemibradyspora*. Comparative studies on species of *Nematosporangium* should be conducted, with as many replications of cultures as possible, to detect variation induced by minor external factors. The writer found that five Petri-dish cultures for each organism at one time on any single test were the fewest that could have been used.

The results obtained on sexual reproduction of different species with the various culture media above mentioned are recorded in Table I.

KEY TO THE SPECIES OF NEMATOSPORANGIUM<sup>9</sup>

- A. Antheridia, 1 to 25, in relation to a single oögonium, narrow, clavate, and agchylolaimic,<sup>10</sup> mostly on long stalks; oöspores, plerotic mostly;  
section: *Polyandra*.

<sup>9</sup> This key is more descriptive than necessary for the purpose of pointing out most of the outstanding morphological and cultural characters of each organism.

<sup>10</sup> agchylolaimic = crooknecked, from the Greek ἀγκύλος = crook and λαιμός = neck.

TABLE I  
PRODUCTION OF OÖSPORES BY DIFFERENT SPECIES OF *Nematosporangium* IN VARIOUS CULTURE MEDIA

Organisms <sup>1</sup>	Oöspore production in days:										
	Culture media										
A	B	C	D	E	F	G	H	I	J	K	L
<i>Polyandra</i>	—	—	—	+5	—	—	—	—	+15	+7	+10
<i>N. arrenomanes</i> .....	—	—	+20	—	—	—	—	—	20	—	—
" var. <i>hawaiiensis</i> .....	—	—	—	—	—	—	—	—	(?)	+7	—
" <i>Branstetter's</i> .....	—	—	—	—	+2	+2	—	—	+2	+2	+3
<i>N. spangiogammon</i> .....	—	—	—	—	+4	+5	+2	—	+10	—	—
<i>N. hypothalostictum</i> .....	—	—	—	+3	+3	+3	+2	+4	+2	+2	+2
<i>N. polystandron</i> .....	—	—	—	—	+3	+3	—	+3	+3	+3	+3
<i>N. thysonophyllum</i> .....	—	+	—	+3	—	+2	+2	+2	+2	+2	+2
<i>N. rhizophilorum</i> .....	—	(?)	+1	+2	+1	+1	+2	+2	+3	—	+1
<i>N. leucostictum</i> .....	—	+	++	++	+1	+1	+1	+1	+1	+2	+2
<i>N. leiohyphion</i> .....	—	+	++	++	+1	+1	+1	+1	+1	+2	+2
<i>N. epiphanozoporon</i> .....	—	(?)	+1	+1	+1	+1	+1	+1	+2	+2	+2
<i>Oligandra</i>	—	—	—	—	—	—	—	—	—	—	—
<i>N. aphaniidermatum</i> .....	+	+	+	+	+	+	+1	+1	+2	+2	+2
<i>N. Butleri</i> .....	—	—	—	—	—	—	—	—	—	—	+4
<i>N. Indigoferae</i> .....	—	—	—	—	—	—	—	—	—	—	+4

<sup>1</sup> The species listed here are described later in this paper.

<sup>2</sup> Production of oöspores indicated by + and nonproduction by — signs. Numbers have been inserted after + signs in some cases indicating the number of days required for their production.

- B.** Organisms reproducing sexually only in a limited variety of culture media and in the tissues of hosts rarely and after a long time; oöspores very few in culture media and tissues of hosts; produced near plasmatoögoses; plasmatoögoses small submerged in the substrata; subsection: *Bradyspora*.
- C. Aerial mycelium well-developed especially in culture media containing sugars; branching hyphae relatively straight.
  - D. Oöspores produced in 5 to 15 days on culture media E, J and K. *N. arrhenomanes*.....(1)
  - DD. Oöspores produced in 10 to 15 days on culture media C and J. *N. arrhenomanes* var. *hawaiiensis*.....(2)
  - CC. Aerial mycelium slightly cespitose; branching hyphae zig-zagged; oöspores produced in 5 to 20 days on culture media J and K. *N. arrhenomanes* var. *Branstetteri*.....(3)
- BB.** Organisms reproducing sexually in 2 to 5 days on highly suitable culture media and on less suitable culture media in 7 to 25 days or never; oöspores produced mostly from plasmatoögoses;
- subsection: *Hemibradyspora*.
  - C. Oöspores produced rarely or never in the tissues of hosts, from few to many on culture media; plasmatoögoses submerged in the substratum, not in aggregates; aerial mycelium slightly to moderately developed; oöspores mostly abortive, produced in 1 to 3 days on culture media F, G, J, K and L; *N. spaniogammon*.....(4)
  - CC. Oöspores many developing independently or from plasmatoögoses on suitable culture media; few in the tissues of hosts; plasmatoögoses (on *Carica Papaya* media) grouping in aggregates and protruding slightly above substratum; aerial mycelium well-developed, mostly marginal.
  - D. Plasmatoögoses (on *Carica Papaya*) small, 0.5 to 2 mm., but numerous; oöspores produced in 3 to 15 days, on F, G, H and J culture media; *N. hyphalosticton*.....(5)
  - DD. Plasmatoögoses (on *Carica Papaya* media) few but large, 2 to 7 mm. in diameter; aerial mycelium mostly marginal; oöspores produced in 1 to 2 days, on D, F, G, I, J, K and L, and on less suitable media after 10 to 15 days from plasmatoögoses; *N. polyandron*.....(6)
  - DDD. Plasmatoögoses (on *Carica Papaya* media) with some aerial mycelium, few and large; aerial mycelium over entire colony; oöspores produced in 1 to 3 days on B, D, G, I, J, K or L, never or seldom produced from plasmatoögoses; *N. thysanophyphalon*.....(7)
- BBB.** Sexual reproduction requiring from 1 to 3 days on all culture media; exception, *N. rhizophoron* in media A and K; oöspores in great numbers intra- and extra-matrical in culture media and in tissues of hosts; plasmatoögoses small not in aggregates and produced in or on the surface of the substratum; subsection: *Tachyspora*.
- C. Oöspores intramatrical, mostly fertile and mostly submerged in the substratum; plasmatoögoses (on *Carica Papaya* media)

- submerged in the substratum; aërial mycelium well-developed in culture media containing sugars; *N. rhizophororum* . . . . (8)
- CC.** Oöspores mostly intramatrical one-third to one-half abortive; plasmatoögoses (on *Carica Papaya* media) protruding above the surface of the substratum as whitish specks; aërial mycelium moderately developed, mostly marginal; *N. leucosticton* . . . . . (9)
- CCC.** Oöspores intramatrical and mostly abortive; plasmatoögoses few, embedded in the substratum; aërial mycelium lacking in all culture media except E, K and L; *N. leiohyphom* . . (10)
- CCCC.** Oöspores extramatrical or mostly on aërial mycelium, nine-tenths abortive; plasmatoögoses very few, small and embedded in substratum; aërial mycelium well-developed and may cover entire colony or margins; *N. epiphanoesporon* . . (11)
- AA.** Antheridia, 1 to 2 and rarely 3 to 4, in relation to a single oögonium; broad barrel-shaped, slightly clavate, few with long and mostly with very short stalks, or in some cases, the stalks entirely lacking; oöspores not filling oögonium; section: *Oligandra*.
- B.** Organisms with most plasmatoögoses developing zoösporangia, reproducing asexually by means of zoöspores very readily in water, grown either on a great variety of culture media or in the tissues of hosts; subsection: *Plethoraocomba*.
- C.** Oöspores numerous produced mostly on aërial mycelium in 1 to a few days, on all culture media; plasmatoögoses numerous, produced in the hyphae of aërial mycelium not in aggregates; aërial mycelium profuse in most culture media especially those containing sugars; hyphae, 5 to 10  $\mu$  in diameter, producing many branches at the tips; zoösporangia developing in a few minutes; *N. aphanidermatum* . . . . . (12)
- CC.** Oöspores not very numerous, produced mostly on aërial mycelium, in 3 to 5 days, on C, D, F, G, J and L; plasmatoögoses not very numerous; aërial mycelium moderately developed.
- D.** Hyphae 5 to 10  $\mu$  in diameter, producing many branches at tips; zoösporangia developing in a few to many minutes; *N. aphanidermatum* var. *hawaiiensis*.
- DD.** Hyphae, 5 to 10  $\mu$  in diameter, thysanoid at tips; zoösporangia developing in a few hours; *N. Butleri*.
- BB.** Organisms with very few atypical plasmatoögoses produced in liquid and solid favorable culture media, but not in the tissues of hosts; seldom developing into zoösporangia; subsection: *Oligocomba*.
- Oöspores numerous on aërial and submerged mycelium; plasmatoögoses on mycelium relatively few; not very prominent; aërial mycelium moderately developed; hyphae, 3 to 5  $\mu$  in diameter, with little branching; zoösporangia developing very seldom with few zoöspores; oögonial stalk bending towards antheridium; *N. Indigoferae*.

## DESCRIPTION OF SPECIES

- (1) **N. arrhenomanes** (Drechsler) comb. nov. described by Drechsler (5).
- (2) *N. arrhenomanes* var. **hawaiensis** var. nov. (FIG. 4).  
(*Pythium arrhenomanes* Drechsler, Phytopathology 18: 873-875, 1928.)

Mycelium intra- and extracellular, in culture media exhibiting a profuse aerial development over the entire colony in young cultures; hyphae irregular, 3 to 6  $\mu$  in diameter (average 3  $\mu$ ); plasmatoögoses as individual units and as aggregates, the latter forming occasionally in old cultures and the former being distributed uniformly in the substratum, individual plasmatoögoses 100 to 400  $\mu$ , aggregates 1 to 15 mm., those produced in culture media very seldom developing into prosporangia, abundant in the tissues of hosts in both young and old infections; zoösporangia produced readily from prosporangia under proper environmental conditions, 25 to 45  $\mu$  in diameter, containing from 4 to 50 biciliate reniform zoöspores; zoöspores formed in 15 to 20 minutes in the zoösporangium, motile for 10 to 30 minutes or possibly longer, then rounding up as subspherical bodies about 10  $\mu$  in diameter which may germinate in a few hours by one germ tube measuring almost 3  $\mu$  in diameter; oögonia subspherical, terminal, very few in cultures of three or more weeks old, mostly near the plasmatoögoses, extremely few elsewhere, 20 to 34  $\mu$  in diameter (average 28  $\mu$ ); antheridia agchylolaimic 5 to 8  $\mu$  in diameter in the distal expanded portion, 10 to 20  $\mu$  in length along curved axis from apex to basal septum, the proximal part more gradually tapering toward delimiting septum to diameter of supporting filament, numerous, from 1 to 15 or possibly more often visible in relation to one oögonium, borne terminally and (quite often) laterally on branches arising from the same hypha, the latter surrounding the oögonium and bearing 2 to 12 antheridia; oöspores subspherical, yellowish mostly, plerotic, about 25  $\mu$  in diameter, and surrounded by a wall about 1.5  $\mu$  in thickness, germinating in a few weeks giving rise to vegetative hyphae which may or may not produce prosporangia depending on environmental conditions, rarely occurring in the tissues of hosts and then only in old infections. (Culture media, such as a decoction of the seeds of *Cannabis sativa* or the juice of the ripe fruits of *Carica Papaya*, are best suited for their development.)

It was obtained from the diseased roots of *Ananas sativus* grown on the island of Oahu of the Hawaiian Archipelago. It

is a root parasite of *Ananas sativus*, *Saccharum officinarum* var. H 109, and Lahaina, *Zea Mays*, *Triticum vulgare*, *Panicum barbinode*, *Musa sapiente*, *Cajanus indicus* var. New Era, *Phaseolus aureus*, *Solanum tuberosum*, and *Ipomoea Batatas*.

(3) *N. arrhenomanes* var. Branstetter (1).

(4) ***N. spaniogamón* sp. nov.** (FIG. 5).

Mycelium intra- and extracellular, in culture media slightly cespitose, exhibiting a moderate aerial development; hyphae irregular, 3 to 6  $\mu$  in diameter (average 4.5  $\mu$ ); plasmatoögoses (on *Carica Papaya* media) as individual units, never as aggregates forming large colonies, distributed more or less uniformly in the substratum, 100 to 1000  $\mu$ , individual hyphae, 10 to 20  $\mu$ , produced in culture media and in the tissues of hosts; zoösporangia produced readily in hanging drop cultures from prosporangia under favorable environmental conditions, 25 to 40  $\mu$  in diameter containing from 4 to 50 biciliate reniform zoöspores; zoöspores formed in 15 to 20 minutes; in the sporangium, motile for 10 to 30 minutes and possible longer, depending on temperature and optimum condition of the surrounding solution, rounding up as subspherical bodies about 12  $\mu$  in diameter which may germinate in a few hours by one or sometimes two germ tubes 2.5 to 3.0  $\mu$  in diameter; oögonia subspherical, terminal, produced in 2 to 4 days, in highly suitable substrata, many proliferating and abortive, 20 to 30  $\mu$  in diameter (average 25  $\mu$ ), production mostly limited to a single crop; antheridia developing very seldom, agchylolaimic, clavate, and mostly narrow, fertilizing only 2 to 10 per cent of the oögonia produced in the substratum, 1 to 8 in relation to a single oögonium, agchylolaimic, width 5 to 8  $\mu$ , length 8 to 12  $\mu$  along curved axis from apex to basal septum, variable in size and shape; oöspores subspherical, yellowish, mostly plerotic, about 25  $\mu$  in diameter and surrounded by a wall 1.5  $\mu$  in thickness, germinating in a few days or weeks giving rise to vegetative hypha which may or may not produce prosporangia, never observed in the tissues of hosts, but produced in culture media prepared from melon seeds, watermelon seeds, papaya juice, etc.

It was obtained from the diseased roots of *Ananas sativus* grown on the island of Oahu of the Hawaiian Archipelago. It is an aggressive root parasite of *Ananas sativus*, *Saccharum officinarum* var. H 109 and Lahaina, *Zea Mays*, *Triticum vulgare*, *Panicum barbinode* and a weak parasite of *Commelinina nudiuscula*.

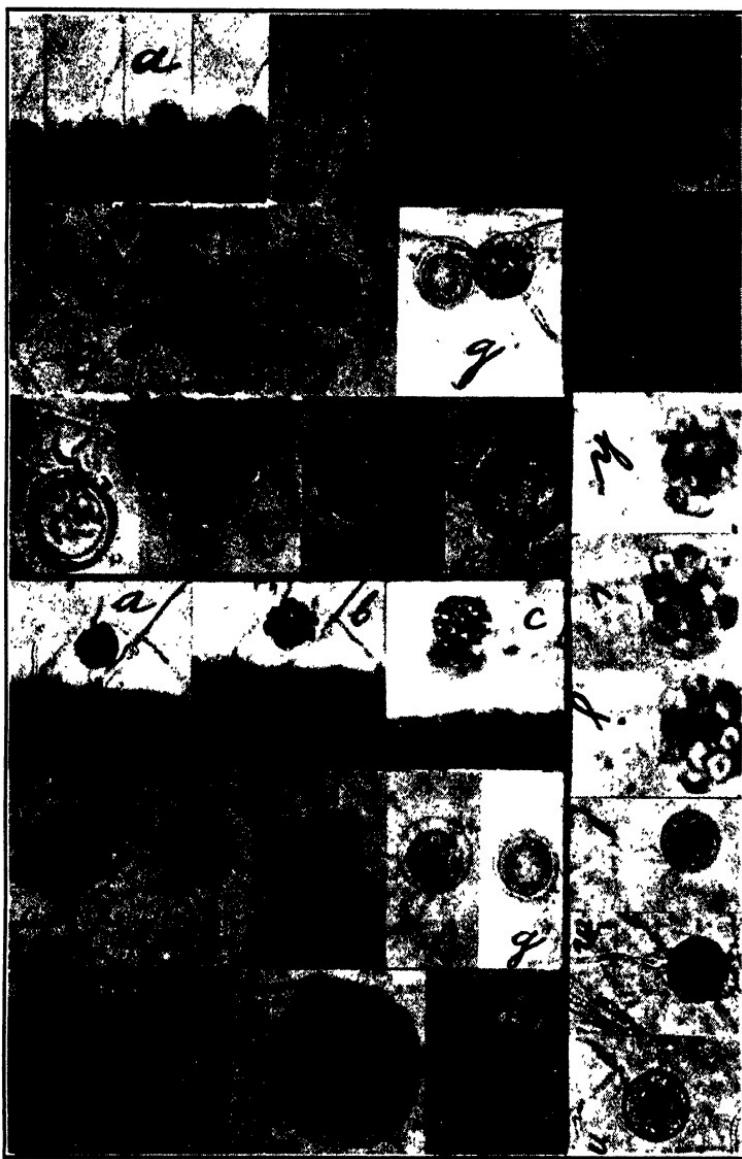


FIG. 4. (right) *N. arrhenomanes* var. *hawaiiensis*. *a, b, c, d, e*, development of zoösporangia ( $\times 300$ ); *h, i, j*, development of the zoösporangia ( $\times 600$ ); *l, m, n*, oöspores ( $\times 300$ ); (upper left) *N. hyphalosticton*. *a*, zoösporangia ( $\times 150$ ); *b*, zoösporangium ( $\times 300$ ); *c*, zoöspore entering a root hair; *d*, germinating zoöspore ( $\times 300$ ); *e*, plasmatoögoses in water; *f, g*, oöspores ( $\times 300$ ); *h, i, j, k*, oöspores ( $\times 600$ ); (lower left) *N. thysanophyphalon*. *a, b, c*, zoösporangia ( $\times 150$ ); *d, e, f, g, j*, oöspores ( $\times 300$ ); *h, i*, oöspores ( $\times 600$ ).

*flora*, *Phaseolus aureus*, *Vicia faba*, *Solanum tuberosum* and *Ipomoea Batatas*.

(5) **N. hyphalosticton** sp. nov. (FIG. 4).

Mycelium intra- and extracellular, in most culture media exhibiting moderate aerial development especially at the margins and occasionally center of the colony; hyphae irregular, 3 to 6  $\mu$  in diameter; plasmatoögoses (on *Carica Papaya* culture media) in many small aggregates 0.5 to 3 mm. in diameter, distributed either sporadically or cycladically on the substratum, developing either into oöspores or prosporangia, abundant in both young and old infections in the tissues of hosts; zoösporangia produced readily when infected tissues of hosts are placed in water, 25 to 45  $\mu$  in diameter, containing 4 to 50 biciliate reniform zoöspores; zoöspores formed in 15 to 20 minutes in the zoösporangium, motile for 10 to 30 minutes or possibly longer, rounding up as subspherical bodies about 10  $\mu$  in diameter which may germinate a few hours later by one germ tube 2.5 to 3.0  $\mu$  in diameter; oögonia subspherical, terminal, relatively abundant on suitable culture media, 28 to 38  $\mu$  in diameter, produced either on the laterals of ordinary hyphae in 3 to 4 days, or on those of plasmatoögoses in 2 to 3 weeks; antheridia agchylolaimic, width 5 to 7  $\mu$ , length 10 to 17  $\mu$ , along curved axis from apex to basal septum, the proximal part more gradually tapering toward delimiting septum to diameter of supporting filament, numerous, from 3 to 15 and possibly more often visible in relation to 1 oögonium, terminal and (quite often) lateral, 2 to 12, on very short branches of the same hypha, the latter surrounding the oögonium; oöspores spherical to subspherical, yellowish, mostly plerotic 32  $\mu$  in diameter and surrounded by a wall about 1.5 to 2.0  $\mu$  in thickness, produced in 3 to 4 weeks within or close to the area occupied by plasmatoögoses and only after the formation of the latter, germinating in a few days or weeks by one or (very seldom) two hyphae which may produce prosporangia immediately if environmental conditions are very suitable, rarely occurring in the tissues of hosts and then only in very old infections, produced on culture media prepared with melon seeds, watermelon seeds, and papaya juice.

It was obtained from the diseased roots of *Ananas sativus* grown on the islands of Oahu, Maui and Molokai of the Hawaiian Archipelago. It is a very aggressive root parasite of *Ananas sativus*, *Saccharum officinarum* var. H 109 and *Lahaina*, *Zea Mays*, *Triticum vulgare*, *Panicum barbinode* and a weak parasite

of *Musa sapiente*, *Cajanus indicus* var. New Era, *Phaseolus aureus*, *Solanum tuberosum* and *Ipomoea Batatas*.

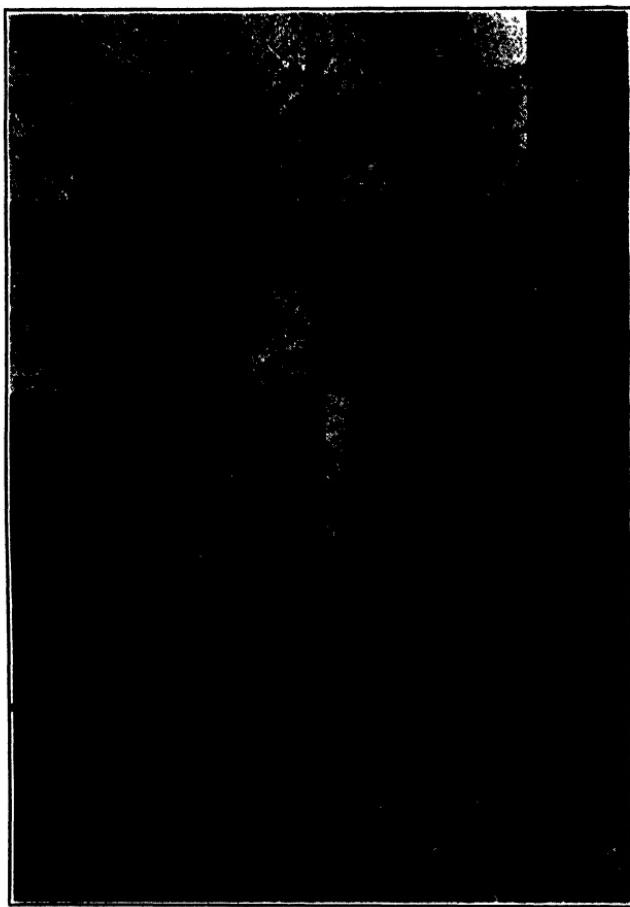


FIG. 5. *N. spaniogamon*. — a, plasmatoögoses in water ( $\times 300$ ); b, zoosporangium ( $\times 150$ ); c, zoospores ( $\times 300$ ); d, oöspore and undeveloped oögonia; e, abortive oögonia; f, abortive oöspore.

(6) ***N. polyandron* sp. nov. (FIG. 6).**

Mycelium intra- and extracellular, in culture media exhibiting moderate aerial development at the margins of the colony; hyphae irregular, 3 to 6  $\mu$  in diameter (average 4  $\mu$ ); plasmatoögoses (on *Carica Papaya* culture media) in large aggregates 3 to 15 mm., few to many in cultures 3 to 4 weeks old, individual units 100 to 400  $\mu$  and individual hyphae from 10 to 25  $\mu$ , abundant in the tissues of hosts of both young and old infections; zoospo-

rangia produced readily from prosporangia under proper environmental conditions, 25 to 45  $\mu$ , containing 4 to 50 biciliate reniform zoospores; zoospores formed in 15 to 20 minutes, motile for 10 to 30 minutes or possibly longer, then rounding up as subspherical bodies about 10  $\mu$  in diameter which germinate in a few hours by one germ tube 2.5 to 3.0  $\mu$  in diameter; oögonia subspherical, terminal, 28 to 38  $\mu$  in diameter (average 33  $\mu$ ); produced in cultures 3 to 4 weeks old, in great numbers near or within the area occupied by plasmatoögoses; antheridia agchyolalimic, 5 to 7  $\mu$  in diameter in the distal expanded portion and 10 to 17  $\mu$  in length along curved axis from apex to basal septum, the rounded apical end making narrow contact with oögonium about a short fertilization tube that measures approximately 2.5  $\mu$  in diameter, the proximal part more gradually tapering toward delimiting septum, to diameter of supporting filament, numerous, from 1 to 15 or possibly more often visible in relation to an oögonium, terminal and (quite often) lateral on very short branches arising from single hypha, the latter surrounding the oögonium and bearing 2 to 12 antheridia; oöspores spherical to subspherical, yellowish, mostly plerotic, produced between plasmatoögoses in 3 to 4 weeks old cultures, about 32  $\mu$  in diameter, wall 1.5 to 2.0  $\mu$  thick, germinating in a few days or weeks by one or very seldom two hyphae which may produce prosporangia immediately if environmental conditions are very suitable, rarely occurring in the tissues of hosts and then only in very old infections, in culture media (hempseeds, melon seeds, watermelon seeds, avocado, papaya, etc.) produced in 2 to 4 days.

It was obtained from the diseased roots of *Ananas sativus* grown on the islands of Oahu, Maui and Molokai of the Hawaiian Archipelago. It is a very aggressive root parasite of *Ananas sativus*, *Saccharum officinarum* var. H 109 and *Lahaina*, *Zea Mays*, *Triticum vulgare*, *Panicum barbinode* and a weak parasite of *Musa sapiente*, *Cajanus indicus* var. New Era, *Phaseolus aureus*, *Solanum tuberosum* and *Ipomoea Batatas*.

(7) **N. thysanohyphalon** sp. nov. (FIG. 4).

Mycelium intra- and extracellular, in culture media exhibiting a profuse aerial development at the margins of the colony; hyphae irregular, 3 to 6  $\mu$  in diameter (average 4  $\mu$ ); plasmatoögoses in large aggregates, 3 to 13 mm., few in number and mostly located at the center of the colony with small amount of aerial mycelium, forming in one week old cultures or slightly older; individual plasmatoögoses measuring from 100 to 400  $\mu$

and their hyphae 10 to 20  $\mu$ ; occurring abundantly in the tissues of hosts mostly completely filling the cells of hosts; zoösporangia produced readily from prosporangia under proper environmental conditions from infected tissues of hosts, 25 to 45  $\mu$  in diameter and containing from 4 to 50 biciliate reniform zoospores; zoöspores formed in 15 to 20 minutes in the zoösporangium, motile for 10 to 30 minutes or possibly longer, then rounding as sub-

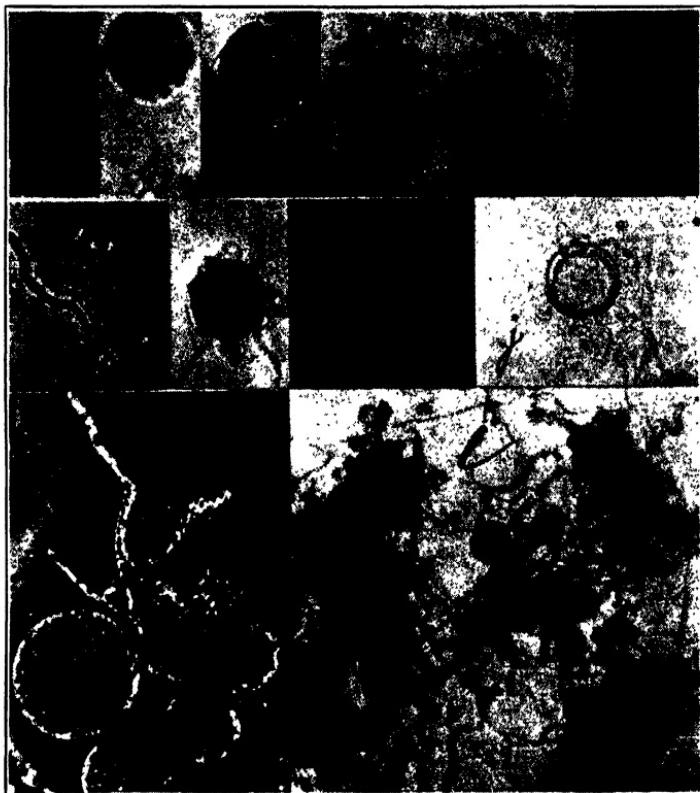


FIG. 6. *N. polyandron*. a, b, c, d, e, f, development of the zoösporangium ( $\times 300$ ); g, zoospore ( $\times 300$ ); h, j, oöspores ( $\times 300$ ); i, oöspores ( $\times 600$ ).

spherical bodies about 10  $\mu$  in diameter which may germinate a few hours later by one germ tube 2.5 to 3.0  $\mu$  in diameter; oögonia subspherical, terminal, produced in great numbers in young cultures and distributed throughout the substratum, 20 to 30  $\mu$  (average 25  $\mu$ ) in diameter; antheridia agchylolaimic, 5 to 7  $\mu$  in diameter in the distal expanded portion and 10 to 17  $\mu$  in length along curved axis from apex to basal septum, the proximal part more gradually tapering toward delimiting septum to

diameter of supporting filament, 1 to 12 or possibly more often visible in relation to an oögonium, terminal and quite often lateral, on very short branches arising from single hyphae, surrounding the oögonium; oöspores spherical to subspherical, yellowish, mostly plerotic,  $25 \mu$  in diameter, produced in young cultures simultaneously with plasmatoögoses and distributed throughout substratum, wall about  $1.5$  to  $2.0 \mu$  in thickness, occurring rarely in the tissues of hosts of very old infections, produced in culture media of Quaker Oats, hempseeds, water-melon seeds, avocado, papaya, etc.

It was obtained from the diseased roots of *Ananas sativus* grown on the island of Molokai of the Hawaiian Archipelago. It is a very aggressive root parasite of *Ananas sativus*, *Saccharum officinarum* var. H 109 and *Lahaina*, *Zea Mays*, *Triticum vulgare*, *Panicum barbinode* and a weak parasite of *Solanum tuberosum* and *Ipomoea Batatas*.

(8) **N. rhizophthonon** sp. nov. (FIG. 7).

Mycelium intra- and extracellular, in culture media exhibiting a moderate aerial development mostly at the margins of the colony; hyphae irregular 3 to  $6 \mu$  in diameter (average  $4 \mu$ ) with laterals terminating in blunt tips or subspherical structures 5 to  $10 \mu$  in diameter; plasmatoögoses (on *Carica Papaya* media) not in aggregates, individual units distributed throughout and submerged by substratum, 100 to  $400 \mu$  and their hyphae from 10 to  $20 \mu$ ; occurring in moderate numbers in the tissues of hosts and may or may not fill completely the host cells, produced in 10-day old cultures and in the tissues of hosts in young as well as old infections; zoösporangia developing readily (in 30 minutes) from prosporangia produced on the infected tissues of hosts under proper environmental conditions, 25 to  $45 \mu$  in diameter, containing from 4 to 50 biciliate, reniform zoöspores; zoöspores about  $10 \mu$  in diameter, at rest, formed in 15 to 20 minutes in the zoösporangium, motile for 10 to 30 minutes or possibly longer, then rounding up as subspherical bodies which may germinate by one, two or three germ tubes 2.5 to  $3.0 \mu$  in diameter; oögonia spherical to subspherical, terminal, produced in great numbers in young cultures and distributed throughout substratum, 28 to  $40 \mu$  (average  $34 \mu$ ); antheridia agchylolaimic, 5 to  $10 \mu$  in diameter in the distal expanded portion and 12 to  $25 \mu$  in length along curved axis from apex to basal septum, the proximal part more gradually tapering toward delimiting septum, to diameter of supporting filament, 1 to 12 or possibly more, often visible in

relation to an oögonium, terminal and quite often lateral; oöspores spherical to subspherical, yellowish, mostly plerotic, 25 to 38  $\mu$  (average 33  $\mu$ ) in diameter and surrounded by a wall about 1.5 to 2.0  $\mu$  in thickness, produced in great number in the substratum and tissues of hosts of young and old infections and mostly at the base of root hairs.

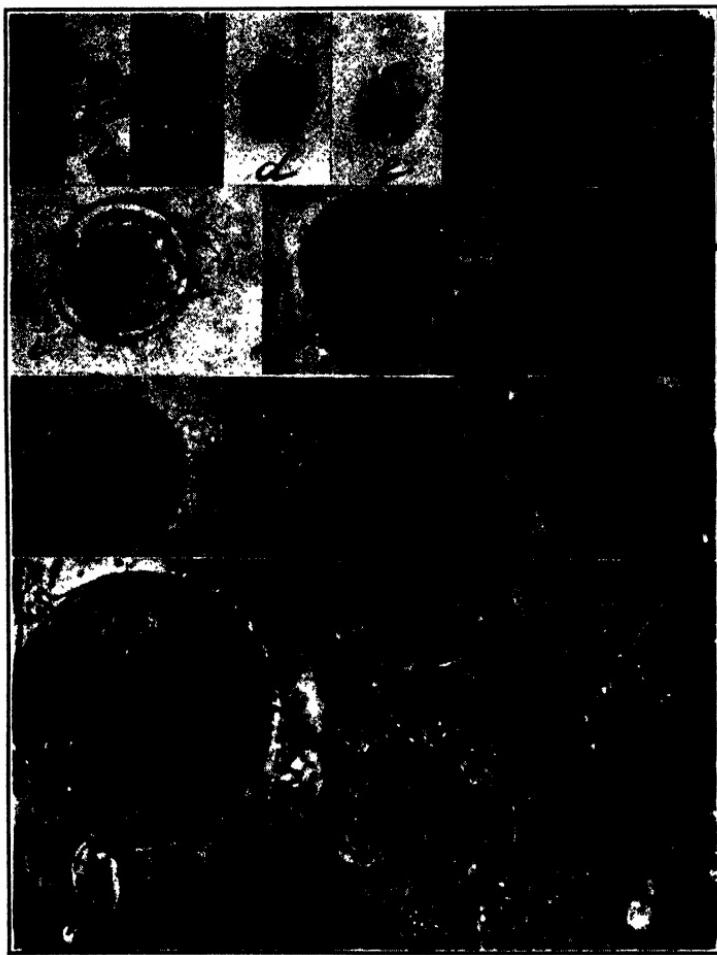


FIG. 7. *N. rhizophthonon*. *a, b, c*, zoosporangia ( $\times 150$ ); *d, e*, zoosporangia ( $\times 300$ ); *f, g, h*, zoospores ( $\times 300$ ); *i, j, k, l, m, n*, oöspores ( $\times 600$ ); *o*, oöspore ( $\times 1050$ ); *p*, plasmatoögoses ( $\times 600$ ).

It was obtained from the diseased roots of *Ananas sativus* grown on the islands of Oahu, Maui, Molokai, Kauai and Lanai of the Hawaiian Archipelago and is one of the most predominant

species in pineapple fields. It is a very aggressive root parasite of *Ananas sativus*, *Saccharum officinarum* var. H 109 and *Lahaina*, *Zea Mays*, *Triticum vulgare*, *Panicum barbinode* and less aggressive on *Solanum tuberosum* and *Ipomoea Batatas*.

(9) **N. leucosticton** sp. nov. (FIG. 8).

Mycelium intra- and extracellular, in culture media exhibiting a moderate aerial development mostly at the margins of the colony; hyphae irregular 3 to 6  $\mu$  in diameter (average 4  $\mu$ ) laterals terminating mostly in subspherical bodies about 20  $\mu$  in diameter devoid of protoplasm (these, in the opinion of the writer, are undeveloped oögonia); plasmatoögoses (on *Carica Papaya* media) not in great aggregates, mostly small, 100 to 400  $\mu$ , distributed throughout substratum, either submerged or protruding above the surface, in the latter case appearing as small white spots occupying mostly the marginal area of the colony, forming in cultures 2 to 3 weeks old, produced also in moderate quantities in the tissues of hosts; zoösporangia developing readily, from prosporangia produced on the infected tissues of hosts under proper environmental conditions, 25 to 50  $\mu$ , containing from 4 to 50 biciliate reniform zoöspores; zoöspores approximately 10  $\mu$  in diameter, at rest, formed in 15 to 20 minutes in the zoösporangium, motile for 10 to 30 minutes or possibly longer, then rounding up as subspherical bodies which may germinate by one germ tube 2.5 to 3.0  $\mu$  in diameter; oögonia subspherical terminal or lateral, 28 to 40  $\mu$ , produced in great numbers, many sterile (failing to produce oöspores even after they become fertilized, remaining as empty spheres and surrounded by 1 to 12 antheridia), produced in one week old cultures and distributed throughout the substratum; antheridia agchylolaimic, 5 to 8  $\mu$  in diameter in the distal expanded portion and 15 to 25  $\mu$  in length along curved axis from apex to basal septum, the proximal part more gradually tapering toward delimiting septum, to diameter of supporting filament, 1 to 12 or possibly more often visible in relation to an oögonium, terminal and quite often lateral; oöspores spherical to subspherical, yellowish, mostly plerotic, 20 to 40  $\mu$  in diameter (average 30  $\mu$ ), surrounded by a wall 1.5 to 2.0  $\mu$  in thickness, produced in great numbers in the tissues of hosts in both young and old infections and in the substratum of cultures about one week old.

It was obtained from the diseased roots of *Bilbergia* sp. grown in the greenhouse of the Experiment Station. It is a very aggressive root parasite of *Ananas sativus*, *Saccharum officinarum*

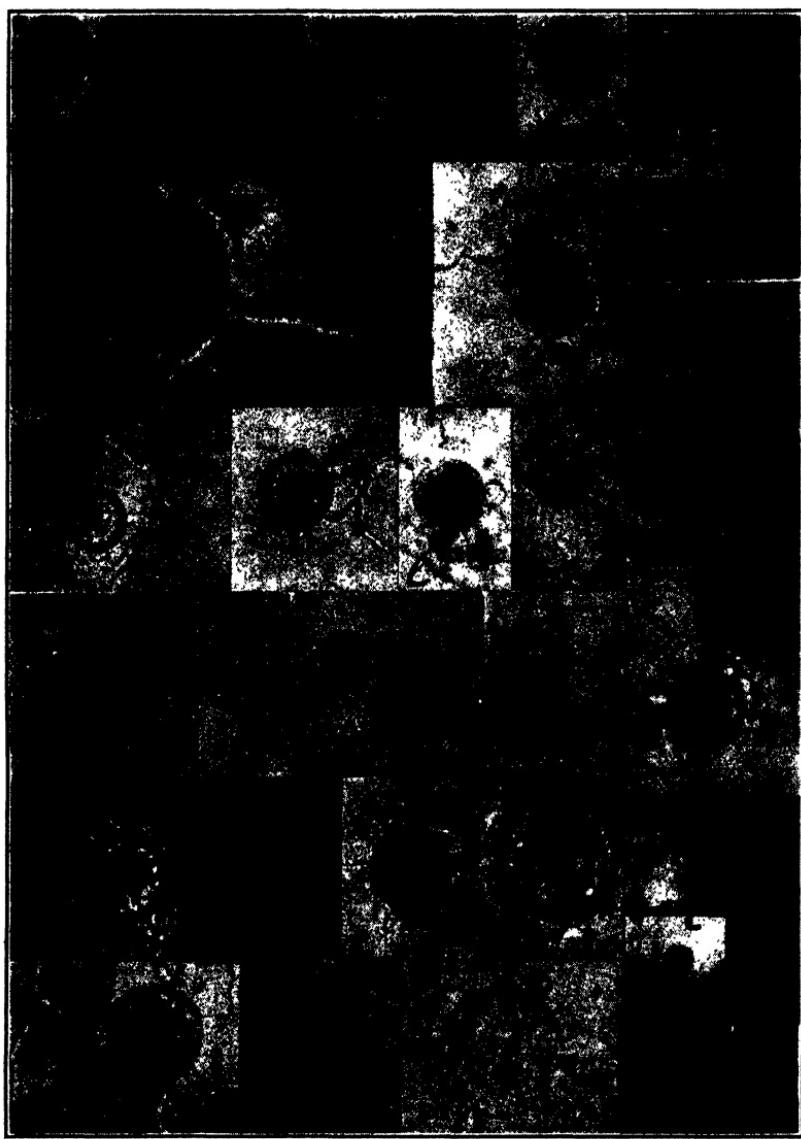


FIG. 8. (right) *N. leucosticton*. *a, c*, zoosporangia ( $\times 150$ ); *b, d*, zoosporangia ( $\times 300$ ); *e, f, g, h, i, j, k, l*, oospores ( $\times 300$ ); *m*, plasmatoogones and two undeveloped oögonia; (lower left) *N. leiohyphon*. *a, b, c, d, e, f, g, h*, development of zoosporangium ( $\times 300$ ); *i, j*, zoospores ( $\times 300$ ); *m, n, p, s, u*, oospores ( $\times 300$ ); *r, l, o, t*, oospores ( $\times 600$ ); *r*, abortive oöspore ( $\times 600$ ); (upper left) *N. epiphanothorpon*. *a, b, c, d, e, f*, development of zoosporangium ( $\times 300$ ); *g, l, n*, oospores ( $\times 300$ ); *h, i, j, m*, oospores ( $\times 600$ ); *k*, oöspore ( $\times 900$ ); *o*, oospores and antheridia ( $\times 300$ ).

var. H 109 and *Lahaina*, *Zea Mays*, *Triticum vulgare*, *Panicum barbinode* and a less aggressive one on *Ipomoea Batatas*.

(10) **N. leiohyphon** sp. nov. (FIG. 8).

Mycelium intra- and extracellular, in culture media exhibiting either a very faint aërial development or lacking it entirely; hyphae irregular 3 to 6  $\mu$  (average 4  $\mu$ ); plasmatoögoses (on *Carica Papaya* media) not forming large aggregates, 100 to 400  $\mu$ , submerged and distributed throughout substratum, produced in 1 to 2 weeks old cultures in moderate quantities and in the tissues of hosts; zoösporangia produced readily, from prosporangia developing on the recently infected tissues of hosts under proper environmental conditions, 25 to 50  $\mu$  and containing from 4 to 50 biciliate reniform zoospores; zoospores motile for 10 to 30 minutes and then rounding up as subspherical bodies about 12  $\mu$  in diameter germinating in a few hours by one or rarely two germ tubes 2.5 to 3.0  $\mu$  in diameter; oögonia subspherical, terminal and rarely intercalary, some sterile, 25 to 38  $\mu$  (average 32  $\mu$ ), wall approximately 0.5  $\mu$  in thickness; antheridia agchylo-laimic, 5 to 8  $\mu$  in diameter in the distal expanded portion and 10 to 20  $\mu$  in length along curved axis from apex to basal septum, the rounded apical end making narrow contact with oögonium about a short fertilization tube that measures approximately 2.5  $\mu$  in diameter, the proximal part more gradually tapering toward delimiting septum, to diameter of supporting filament, 2 to 15 or possibly more often visible in relation to an oögonium; terminal and quite often lateral on branches arising from the same hypha and numbering from 2 to 12; oöspores (on *Carica Papaya* agar or tissues of hosts) subspherical, yellowish, mostly plerotic, 25 to 38  $\mu$  (average 32  $\mu$ ) in diameter, containing a reserve globule often 12 to 19  $\mu$ ; wall 1.2 to 2.0  $\mu$  in thickness; produced in one-week-old cultures and in the tissues of hosts of young as well as old infections.

It was obtained from the diseased roots of *Ananas sativus* grown on Oahu. It is an aggressive root parasite of *Ananas sativus*, *Saccharum officinarum* var. H 109 and *Lahaina*, *Triticum vulgare*, *Zea Mays*, *Panicum barbinode* and a less aggressive one on *Solanum tuberosum*.

(11) **N. epiphano sporon** sp. nov. (FIG. 8).

Mycelium intra- and extracellular, in culture media exhibiting a very profuse aërial development over the entire colony; hyphae irregular about 4  $\mu$  and may vary between 3 and 5  $\mu$ ; plasma-

toogoses (on *Carica Papaya media*) small and few mostly embedded in the substratum and almost indistinguishable by the naked eye, developing in one-week-old cultures, produced either simultaneously with or slightly after the sexual organs, developing into prosporangia or germinating as conidia depending on environmental conditions, occurring in the tissues of hosts in moderate numbers; zoösporangia developing readily in water from prosporangia produced on portions of recently-infected root tissues of hosts, 25 to 60  $\mu$  and containing from 4 to 50 and possibly more biciliate reniform zoöspores; zoöspores motile for 10 to 30 minutes and then rounding up as subspherical bodies, about 10  $\mu$  in diameter, which may germinate in a few hours by one or rarely two germ tubes 2.5 to 3.0  $\mu$  in diameter; oögonia (on *Carica Papaya* agar) subspherical, terminal and rarely intercalary, produced on the aerial mycelium and rarely embedded in the substratum, many sterile, 20 to 40  $\mu$  (average 30  $\mu$ ) with a wall about 0.5  $\mu$  in thickness, produced in great numbers in the tissues of hosts; antheridia agchylolaimic, 5 to 8  $\mu$  in diameter in the distal expanded portion and 10 to 20  $\mu$  in length along curved axis from apex to basal septum, the proximal part more gradually tapering toward delimiting septum to diameter of supporting filament, numerous from 2 to 8 or possibly more often visible in relation to an oögonium, terminal and sometimes lateral; oöspores subspherical, yellowish mostly plerotic, 20 to 40  $\mu$  (average 30  $\mu$ ), containing a reserve globule and surrounded by a wall 1.2 to 2.2  $\mu$  in thickness, produced in relatively great numbers in the tissues of hosts.

It was obtained from the diseased roots of *Ananas sativus* grown on the island of Oahu of the Hawaiian Archipelago. It is an aggressive root parasite of *Ananas sativus*, *Saccharum officinarum* var. H 109 and *Lahaina*, *Zea Mays*, *Triticum vulgare*, *Panicum barbinode* and a less aggressive one on *Solanum tuberosum*.

(12) *N. aphanidermatum* (Edson) Fitzpatrick (FIG. 9).

(*Rheosporangium aphanidermatum* Edson, Jour. Agr. Res. 4: 279-291. 1915.)

*Pythium aphanidermatum* Fitzpatrick; (*Nematosporangium aphanidermatum* Fitzpatrick, Mycologia 15: 166-173. 1923.)

Mycelium intra- and extracellular, in culture media exhibiting a very profuse aerial development and extremely rapid growth (the colony being able to make a growth 100 mm. in diameter in

48 hours); hyphae irregular, those of the aerial mycelium producing many branches at the tips, showing false dichotomy measuring from 3 to 8  $\mu$  in diameter and occasionally septate



FIG. 9. *N. aphanidermatum* (Edson) Fitzpatrick. a, b, c, d, e, development of zoösporangium ( $\times 300$ ); f, h, zoöspores germinating ( $\times 300$ ); g, plasmatoögoses on aerial mycelium; i, oöspores ( $\times 300$ ); j, oöspores ( $\times 600$ ).

in old cultures; plasmatoögoses in great number on the aerial mycelium, not forming aggregates and occasionally embedded in the substratum, in the tissues of hosts forming in moderate

quantities; zoösporangia forming readily from prosporangia in hanging-drop cultures, at temperatures between 25° and 30° C, measuring on an average 45  $\mu$  and containing 4 to 60 biciliate reniform zoöspores; zoöspores produced in the zoösporangium in 15 to 20 minutes, motile for 10 to 30 minutes then rounding up as subspherical bodies 8 to 12  $\mu$  in diameter which may germinate in a few hours by a single germ tube 2.5 to 3.0  $\mu$  in diameter; oögonia very numerous, on most substrata borne laterally on very short budlike branches but may be intercalary on the aërial mycelium (seldom on the submerged mycelium), subspherical, thin-walled, 20 to 30  $\mu$  (average 25  $\mu$ ) in diameter; antheridia terminal or intercalary doliform or broadly clavate, 9 to 11  $\mu$  in diameter in the distal expanded portion and 10 to 14  $\mu$  in length along axis from apex to basal septum, supporting filament very short 3 to 6  $\mu$  in length and occasionally slightly longer, 1 to 2 and rarely 3 to 4 visible in relation to an oögonium; oöspores spherical to subspherical, single, aplerotic with a reserve globule, wall 1.0 to 2.0  $\mu$  in thickness, produced in greater numbers on the aërial than submerged mycelium, and in moderate numbers in the tissues of hosts.

It was first obtained from seedlings of *Beta vulgaris* by Edson and since isolated from many other plants. It is a root parasite of many plants including *Ananas sativus*.

(13) *N. aphanidermatum* var. **hawaiensis** var. nov. (FIG. 10).

This organism differs from *N. aphanidermatum* (Edson) Fitzpatrick in the less vigorous production of aërial mycelium, the slightly branched tips of the aërial hyphae, the slower and rarer production of oöspores and of zoösporangia from plasmatoögoses. It was found on the diseased roots of *Carica Papaya* grown in the writer's garden in Manoa Valley, in the City of Honolulu.

(14) **N. Butleri** (Subram.) comb. nov. (FIG. 11).

(*Pythium Butleri* Subram. Mem. Dept. Agr. India, Bot. Ser. 10: No. 4. 1919.)

Mycelium intra- and extracellular, in most culture media exhibiting a profuse aërial development and a relatively rapid growth; hyphae irregular, those of the aërial mycelium often producing thysanoid growth at their tips, with false dichotomy, 3 to 8  $\mu$  in diameter and frequently septate in old cultures; plasmatoögoses in great number on the aërial mycelium, never

uniting to form aggregates, and occasionally embedded in the substratum and tissues of hosts, developing often into prosporangia; zoösporangia forming in 2 hours from prosporangia in hanging drop cultures, at temperatures between 25° and 30° C, measuring on an average 45  $\mu$  (ranging between 20 and 120  $\mu$ )

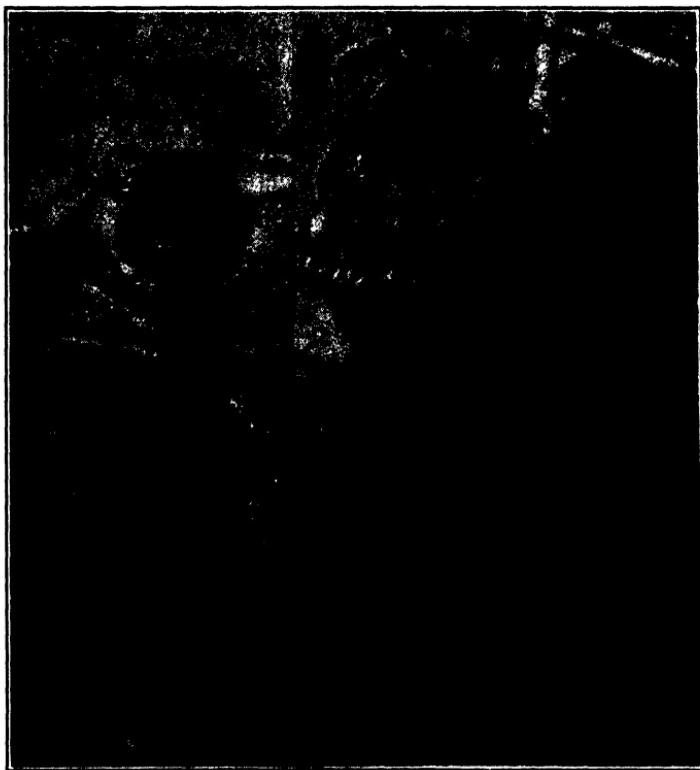


FIG. 10. *N. aphanidermatum* var. *hawaiiensis*. Sexual organs ( $\times 600$ ).

and containing 4 to 60 biciliate reniform zoöspores; zoöspores produced in the zoösporangium in 15 to 20 minutes, motile for 30 minutes then rounding up as subspherical bodies 8 to 12  $\mu$  in diameter which may germinate in a few hours by a single germ tube; oögonia not very numerous, produced in 3 to 7 days on most substrata, lateral and intercalary on very short budlike branches or longer branches of the aerial mycelium and occasionally on the embedded mycelium, subspherical and thin-walled, 20 to 30  $\mu$  in diameter; antheridia terminal or on very short laterals broadly clavate, 9 to 11  $\mu$  in diameter in the distal expanded portion and 10 to 14  $\mu$  in length along axis from apex

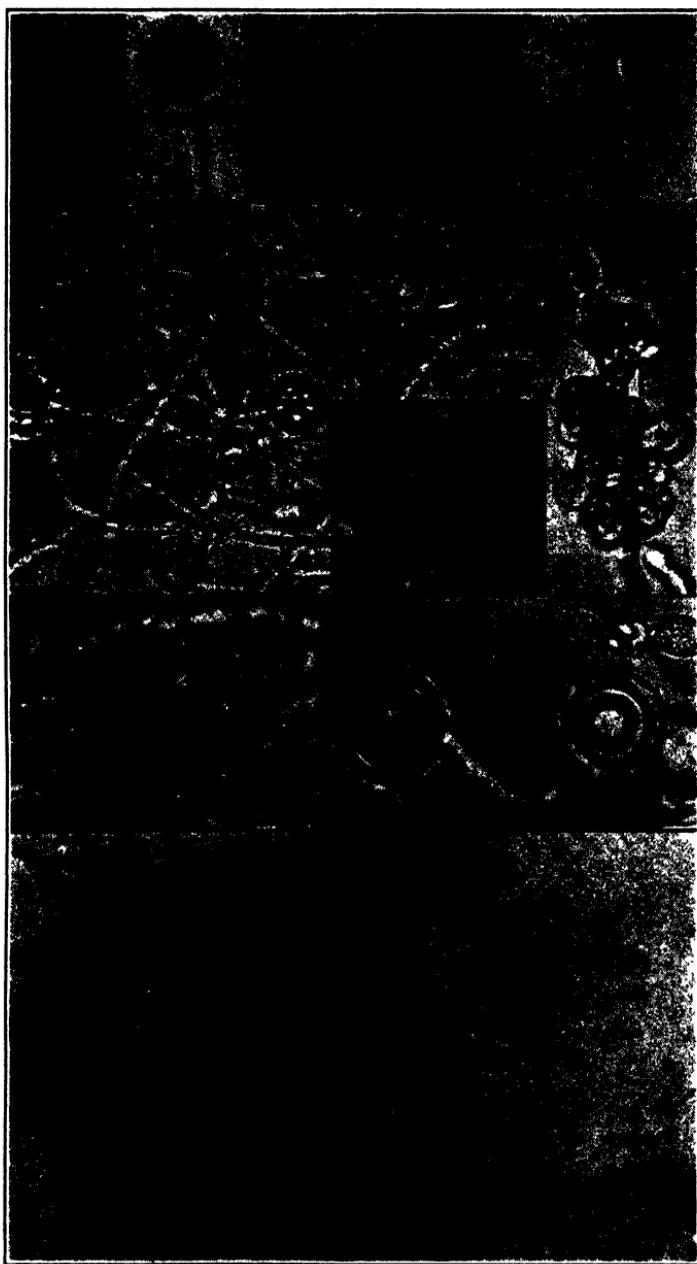


FIG. 11. *N. Butleri* (Subramanian). *a, b, c, d*, development of zöosporangium ( $\times 300$ ); *e, f*, zöospores germinating ( $\times 300$ ); *g*, plasmatoögoses on aerial mycelium; *h*, oöspores ( $\times 300$ ); *i, j, k, l*, oöspores ( $\times 600$ ); *m*, tip of aerial hypha-thsanoid structure.

to basal septum; supporting filament mostly short 3 to 6  $\mu$  in length and occasionally slightly longer, 1 to 2 and seldom 3 visible in relation to an oögonium; oospores spherical to sub-spherical, single, not numerous, aplerotic, wall 1.0 to 2.0  $\mu$  in thickness; not very numerous in the tissues of hosts or various culture media.

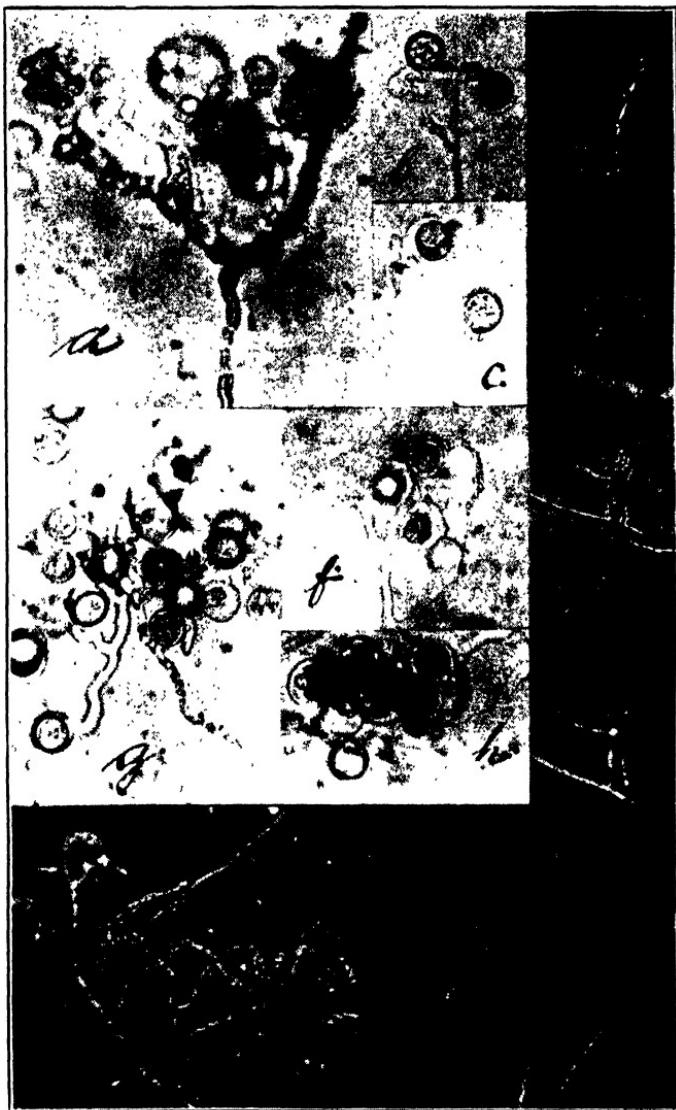


FIG. 12. *N. Indigoferae* (Butler). *a*, oospores ( $\times 600$ ); *b*, *c*, *d*, *e*, *f*, *g*, *h*, oospores ( $\times 300$ ); *i*, *j*, plasmatoögoses on aerial mycelium.

It was obtained by Subramanian from the diseased roots of *Carica Papaya*, *Zingiber officinalis* and *Nicotiana Tabacum* grown in India. It is a root parasite of many plants including *Ananas sativus*.

(15) ***N. indigoferae*** (Butler) comb. nov. (FIG. 12).

(*Pythium Indigoferae* Butler, Mem. Dep. Agr. India 1: No. 5. 1907.)

Mycelium intra- and extracellular, in culture media exhibiting a moderate to faint aerial development; hyphae irregular in size 3 to 5  $\mu$  in diameter; plasmatoögoses in moderate numbers on the aerial mycelium, not forming aggregates and rarely, if ever, developing into prosporangia, but instead into sexual organs; zoösporangia, developing very seldom, and according to Butler (2) very small, opening laterally by short straight branches, producing from 4 to 20 zoöspores; zoöspores about 10  $\mu$  in diameter, at rest, reniform and biciliate; oögonia terminal mostly on rather short lateral branches or in the lateral outgrowths of plasmatoögoses, very constant in size, 18 to 20  $\mu$  in diameter, oögonial stalk or supporting filament strongly curved towards the antheridium producing a characteristic appearance; antheridia cylindrical or doliform, straight, 4 to 7  $\mu$  in diameter and 8 to 12  $\mu$  in length along axis from apex to basal septum, terminal or on very short laterals supporting filament in the latter either lacking entirely or extremely short; oöspores spherical to subspherical, aplerotic smooth 10 to 16  $\mu$  in diameter, germinating rapidly by a hypha, not by zoöspores.

It was obtained from the leaves of *Indigofera arrecta* Hochst., by Butler and by McRae<sup>11</sup> from the roots of *Cucumis sativus* in India. It is a saprophyte but may under certain conditions enter the tip of the roots of *Ananas sativus*.

#### DISCUSSION

Schröter doubtless raised *Nematosporangium* to generic rank because of the morphological differences between the prosporangia of the two genera *Pythium* and *Nematosporangium*, which differences are of fundamental importance. If we were dealing with only one or two species of *Nematosporangium* then we might have been justified in permitting these few distantly-related

<sup>11</sup> This organism was sent to the writer by Dr. W. McRae of the Agricultural Research Institute, Pusa, India.

organisms to remain in the genus *Pythium*. But, as long as we have more than a dozen and as long as these organisms fall into a number of subgroups and require further subdivision for their identification, our non-recognition of the generic rank of *Nematosporangium*, besides being unscientific, tends to cause confusion and stop our further progress.

The morphological features of the genus *Nematosporangium* have been discussed quite extensively in the preceding paragraphs. As morphological differences in the shape and size of oögonia, oöspores, antheridia, prosporangia or zoospores between species of the same section are almost insignificant, rarely exceeding those of normal variation, the adoption of such characters for differentiation would have been misleading. Physiological and certain morphological differences, however, have been found to be fairly constant as well as stable in certain culture media and for this reason they have been adopted for the differentiation and taxonomic classification of the various species. It is rather difficult, if not impossible, to explain satisfactorily the behavior of the different species of *Nematosporangium* on the various culture media, and especially *Carica Papaya* used in these studies. As other organisms such as various species of *Pythium*, *Phytophthora*, and members of Fungi Imperfecti, such as *Fusarium*, *Verticillium*, etc., have been found to make an excellent and normal growth on *Carica Papaya*, the explanation is that, in respect to *Carica Papaya*, it incorporates all the essential food substances for the normal growth of these organisms. It has been found that very dilute concentrations of *Carica Papaya* juice will not produce normal growth in the different fungi. Temperature differences between 22° and 32° C. influence but slightly the physiological behavior of species of *Nematosporangium*. Low temperature may retard and high temperature may accelerate the rate of growth but the differentiating features of the various organisms are always produced.

The various sections have been created to facilitate the grouping of organisms according to certain differences existing in their sexual reproduction. The section *Polyandra* differs from *Oligandra* in the great number of antheridia that are found in relation to a single oögonium. As many as twenty-five antheridia

have been observed by Drechsler on *N. arrhenomanes*. Moreover, the antheridia of the section *Polyandra*, borne either terminally or laterally, are supported on a fairly long stalk, ranging from one to many times the length of the antheridium proper. The section *Oligandra* is characterized by the small number of antheridia, one or sometimes two, in relation to a single oögonium. The antheridia of the section *Oligandra* differ in shape considerably from those of the section *Polyandra*. They are bud-like or barrel-shaped with very slight tapering or none, and the supporting hypha is either very short or almost obsolete. The diameter of the antheridium in its relation to the length is rather great. Other morphological differences between the two sections may be found in the oöspores. The oöspores of all the members of the section *Polyandra* are considerably larger in diameter and mostly fill the oögonium, whereas, those of the section *Oligandra* are about 8 to 10  $\mu$  smaller in diameter and never or very rarely fill the oögonium. Besides, the organisms of the section *Oligandra* produce oöspores and prosporangia mostly on the aerial mycelium or surface of the substratum, whereas, those of the section *Polyandra* produce them mostly in the substratum, except in *N. epiphanoспорон*, which produces them in and on the substratum.

The section *Polyandra* is divided into three subsections, namely, *Bradyspora*, *Hemibradyspora* and *Tachyspora*. The members of the first and second subsections require special culture media, such as juice of the ripe fruit of *Carica Papaya* or a decoction of the seeds of various plants and these in sufficient concentration for their sexual reproduction. Those of the third, however, reproduce sexually in a greater variety of culture media than the former. The organisms of the subsections *Bradyspora* and *Hemibradyspora* reproduce sexually very rarely or never or only in old infections in the tissues of hosts, the probable explanation thereof being that such tissues do not contain food substances in sufficient concentration to enable the rather wasteful process of sexual reproduction of these organisms. Or, it may be that they have to build up sufficient protoplasmic matter for their sexual reproduction and such a process requires very long time, this explaining the production of sexual organs only in old infec-

tions and cultures. The members of the subsection *Tachyspora* reproduce sexually very readily in the tissues of hosts, their oöspores being embedded usually in the exodermal layer of cells of roots. Members of the two subsections may be easily differentiated in the tissues of hosts merely by the presence or absence of oöspores. Between members of the subsection *Tachyspora*, their identification may be made on the relative number of oöspores in the tissues of hosts. *N. rhizophthonon* produces many well-developed and fertile oöspores and likewise many plasmatoögoses. *N. leucosticton* produces fewer oöspores, some of them abortive, and fewer plasmatoögoses. *N. leiohyphon* produces very few oöspores, mostly abortive, and extremely few plasmatoögoses. *N. epiphano sporon* may produce many oöspores but all of them are abortive, there may be an exception occasionally and very few plasmatoögoses. *N. rhizophthonon* is the most predominant root parasite in pineapple fields, followed by *N. polyandron* and *N. hyphalosticton*. The writer is of the opinion that the wide distribution of *N. rhizophthonon* is due to its ability to produce many fertile oöspores.

The section *Oligandra* is divided into the subsections *Plethora-comba* and *Oligo comba*, the former including those members with many plasmatoögoses and the latter those with very few. The members of *Plethora-comba* produce zoösporangia from their plasmatoögoses quite readily in water, indicating that they are hydrobiotic organisms, whereas those of *Oligo comba* produce them very rarely indicating their aerial or terrestrial habitat. The production of oöspores by members of *Oligo comba* is considerably more abundant than by those of *Plethora-comba*, this being an additional evidence of the influence of habitat on the reproduction of the two different groups.

The morphological characteristics of the colonies of different species in the same subsection cannot be attributed to anything else than the inherent physiological behavior of each organism. These differences, constituting a fairly reliable criterion of differentiation between closely related species, are only produced in *Carica Papaya* agar media. The differentiation of species on the basis of a summation of all characters can be easily made out, however, by growing the organisms in the various culture media above-mentioned.

A brief analysis of the ontogenetic and phylogenetic development of the different species of *Nematosporangium* indicates that the members of the section *Oligandra* are more primitive and therefore more elementary in behavior than those of the section *Polyandra*. The evidence for such a characterization of the members of the section *Oligandra* is based on morphological characters such as (1) the great abundance of plasmatoögoses developing mostly into prosporangia on the aërial mycelium, (2) the aplerotic type of oöspores and (3) the rudimentary or primitive type of antheridium. The plasmatoögoses of members of the *Oligandra* except those of *N. Indigoferae* may all develop into prosporangia under favorable conditions and their protoplasm be converted entirely into zoospores, whereas those of section *Polyandra* do not develop into prosporangia as readily, but conserve their protoplasm for vegetative propagation, a safer method under slightly adverse conditions. Such differences reveal that the members of the section *Oligandra* except *N. Indigoferae* are aquatic forms, whereas those of the section *Polyandra* are terrestrial forms.

The writer wishes to acknowledge his indebtedness to Dr. A. L. Dean and Dr. G. H. Godfrey for reading the manuscript and to express his thanks to Dr. J. T. Barrett of the University of California for the use of his laboratory for part of this work and to Mr. G. E. Paxton and Mrs. M. W. Lorimer for technical assistance.

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## NOTES ON NEW SPECIES OF USTILAGINALES<sup>1</sup>

GEORGE L. ZUNDEL.

The descriptions of the following ten new species and one new combination are based on specimens sent to the author by members of the Union Department of Agriculture, Pretoria, Union of South Africa, and by Ross W. Davidson of the office of Mycology and Disease Survey, United States Department of Agriculture. The specimens from the United States Department of Agriculture are from collections made by Mrs. Agnes Chase in Brazil and she has determined all hosts originating from Brazil.

### ***Ustilago brasiliensis* sp. nov.**

Sori spherical, covered with a dark membrane, entirely destroying the ovaries, having the general appearance of a panicle of ripe seeds; about 1 mm. in diam.; spore-mass olive green; spores regular, globose, usually 8  $\mu$  diam., sometimes up to 10  $\mu$ , reddish brown, under oil immersion abundantly papillate.

On *Panicum rivulare* Trin.: Viçosa, Minas Geraes, Brazil; coll. Agnes Chase, April 11, 1925 (U. S. Dept. Agr. Myc. Coll. No. 60382).

### ***Ustilago gregaria* sp. nov.**

Sori in groups along the side branches of the panicle, completely destroying the ovaries which are swollen, globose, about 2 mm. diam., covered with a dark membrane which upon rupturing reveals a dark olive brown spore-mass; spores regular, globose, deep olive brown, usually 6–8  $\mu$  diam., occasionally 10  $\mu$ ; under oil immersion abundantly echinulate, sometimes guttulate.

On *Panicum rivulare* Trin.: Juiz de Fora, Minas Geraes, Brazil; coll. Agnes Chase. February 1925. (U. S. Dept. Agr. Myc. Coll. No. 60384.)

<sup>1</sup> Contribution from The Department of Botany, The Pennsylvania State College No. 75.

**Farysia Pseudocyperi** (Sacc.) comb. nov.

*Ustilago olivacea* forma *Pseudocyperi* Sacc., Syll. 7: 463. 1888.

Sori entirely destroying the ovary, converting it into an olive brown dusty mass of shreds and spores; spores borne between sterile elators or shreds, globose-subglobose, occasionally oblong, olive brown with a distinct thick outer dark olive brown exospore, under oil immersion minutely verruculose, 6–10  $\mu$  diam.

On Cyperaceae (undet. genus); Sin Koong, North Kwangtung, China; coll. F. A. McClure, January 10, 1926. (Unnumbered specimen from U. S. Dept. Agr. Myc. Coll.)

This species differs from *Farysia (Ustilago) olivacea* in having larger spores. It has been previously reported from Argentina on *Carex Pseudo-cyperus* L.

**Sphacelotheca Chaseae** sp. nov.

Sori destroying the individual florets along the rachis of the spike, 4–5 mm. long, covered with a delicate hyaline membrane which dehisces exposing an agglutinated mass of dark brown spores surrounding a columella; cells of the sterile tissue not easily separated; spores 4–6  $\mu$  diam., globose-subglobose, gullulate; light reddish brown; under oil immersion smooth.

On *Mesosetum ferrugineum* (Trin.) Chase; Serra do Cipo, Minas Geraes, Brazil; Coll. Agnes Chase, March 28 and April 1, 1925. (U. S. Dept. Agr. Myc. Coll. No. 60374 and No. 60377.)

**Sphacelotheca brasiliensis** sp. nov.

Sori in the ovaries not concealed by the glumes, 2 mm. long, covered with a delicate yellowish membrane; sterile cells singly, in chains or in groups; globose or irregular due to compression; spores globose-subglobose, light reddish brown, 8–12  $\mu$  diam., under oil immersion minutely but abundantly echinulate.

On *Andropogon leucostachys* H.B.K.: Serra do Cipo, Minas Geraes, Brazil; Coll. Agnes Chase, March 28 and April 1, 1925. (U. S. Dept. Agr. Myc. Coll. No. 60376.)

Of the other species described as occurring on this host, *viz.* *Sphacelotheca leucostachys* (P. Henn.) Zundel; *Sphacelotheca Kelermanii* Clinton & Zundel; and *Sphacelotheca Holwayi* Clinton & Zundel, this species differs in the small sorus, lighter colored and smaller spores.

**Sphacelotheca echinata** sp. nov.

Sori destroying the inflorescence, about 1 cm. long, globose, covered with a dark olive, thick, false membrane which upon maturity ruptures disclosing a brown spore-mass surrounding a columella, false membrane more or less persistent, not easily separated into sterile cells; large sterile cells, about the size of the spores, scattered throughout the sorus; spores globose-subglobose, guttulate, 10–14  $\mu$  diam.; under oil immersion abundantly echinulate.

On *Panicum demissum* Trin.: Serra do Caparaó, Brazil; Coll. Agnes Chase, April 20–May 4, 1925. (U. S. Dept. Agr. Myc. Coll. No. 60371.)

On *Panicum missionum* Mez.: Campos do Jordão, Serra Mantiqueira, Brazil; Coll. Agnes Chase, May 20–22, 1925. (U. S. Dept. Agr. Myc. Coll. No. 60358.)

**Sphacelotheca Mesoseti** sp. nov.

Sori in the inflorescence, destroying the ovary about 3 mm. long, usually all ovaries on an infected spike destroyed, covered with a delicate membrane which breaks up into single or chains of sterile cells, sterile cells globose-subglobose or linear, globose cells 10–14  $\mu$  diam.; spores reddish brown, globose-subglobose, 10–14  $\mu$  diam.; under oil immersion smooth with a delicate epispore that is easily ruptured.

On *Mesosetum loliiforme* (Hochst.) Chase: Parafuso, Bahia, Brazil; Coll. Agnes Chase, Dec. 22, 1924. (U. S. Dept. Agr. Myc. Coll. No. 60389.)

**Sphacelotheca Vryburgii** sp. nov.

Sori in the inflorescence, at first hidden by the glumes but later exposed, long linear 5–10 mm. long, covered by a reddish brown, delicate false membrane which flakes away revealing an agglutinated mass of black spores surrounding a well developed, much branched columella; sterile cells hyaline globose, usually in groups, variable in size, 9–15  $\mu$  diam.; spores globose-subglobose occasionally angled, very light reddish brown, 4–8  $\mu$  diam.; under oil immersion smooth, contents finely granular with a hyaline-like light colored wall.

On *Themedia Forskallii* Kunth.: Vryburg, British Bechuanaland, Union of South Africa; Coll. I. B. Pole Evans, May 5, 1916. (Union Dept. Agr. South Africa, Myc. Herb. No. 9733.)

**Cintractia dubiosa** sp. nov.

Sori in the ovary at first completely hidden by the glumes but later the tip partly protruding, spherical, very firmly agglutinated, hard; spores reddish-brown, with a thick dark reddish epispore but lighter in the center, globose-subglobose, often angular, apparently smooth but under oil immersion minutely papillate,  $12-14 \mu$  diam.

On *Pennisetum* sp.: From Nairobi, British East Africa; Coll. H. L. Shantz, September 9, 1920. (Unnumbered specimen U. S. Dept. Agr. Myc. Coll.)

It is usually considered that the hosts of *Cintractia* are confined to the Cyperaceae. The author is very dubious about placing the above-named species in *Cintractia*. Every character, however, seems to indicate that this species is a *Cintractia* and until more detailed studies are possible this species will be provisionally placed in the genus *Cintractia*.

**Tilletia Paspali** sp. nov.

Sori destroying the ovaries, about 1 mm. long, covered by a delicate membrane which ruptures transforming the entire inflorescence into a black "smutty" mass; spores reddish brown, regular, globose-subglobose, often guttulate,  $18-22 \mu$  diam.; under oil immersion abundantly echinulate.

On *Paspalum millegrana* Schrad.: Matta de São João, Bahia, Brazil; Coll. Agnes Chase, January 3, 1925. (U. S. Dept. Agr. Myc. Coll. No. 60385.)

**Tilletia transvaalensis** sp. nov.

Sori in the ovaries, about 1 mm. long, at first concealed by the glumes but later the tip protrudes slightly, infected spikelets scattered throughout the panicle. Spores regular, globose-subglobose, ranging from a yellowish to a reddish-brown color,  $20-26 \mu$  diam., under oil immersion very prominent, large, echinulate. Sterile cells hyaline, usually smaller than the spores.

On *Eragrostis aspera*: Mucklenburg, Zebediela district, Transvaal, Union of South Africa, Coll. G. W. Wearing, June 6, 1913. (Union Dept. Agr. Myc. Herb. No. 25463.)

## A LIST OF DISEASES OF ECONOMIC PLANTS IN ALABAMA

W. L. BLAIN

Almost three decades have elapsed since anything has been written concerning the fungi of Alabama. Because of the very large number of fungi that have been reported since that time and the increasing interest of the people in this group of plants it has seemed most desirable to present this information in permanent form so as to be available to the people of Alabama, as well as to other workers in the same field outside the state.

The early knowledge of the extent and distribution of fungi in the state is due to the efforts of T. M. Peters and John T. Beaumont. Their collections were identified and described by M. J. Berkeley and M. A. Curtis in the first to third volumes of *Grevillea* (1872 to 1876) under the title of "Notice of North American Fungi." Some of Peters' earlier collections were sent to H. W. Ravenel and were distributed in Ravenel's *Fungi Caroliniana Exsiccati* (1852 to 1860).

Nothing more was done toward making known the mycological flora of the state until G. F. Atkinson came to the state in 1889. Atkinson added considerably to the list of parasitic forms, publishing several papers on fungi during his stay in the state and after going to Cornell University. His field work was confined chiefly to the immediate territory of the college and the results of this study were published in Cornell University Bulletin, volume 3, number 1, 1897.

Atkinson was succeeded by L. M. Underwood and F. S. Earle. They devoted considerable time to collecting fungi and covered a rather extensive portion of the state. The result of their work, "A Preliminary List of Alabama Fungi," was published as Bulletin 80 of the Alabama Experiment Station in 1897.

All of the fungi collected in the state up to and including those mentioned in the publications of Underwood and Earle are included in "Plant Life of Alabama," by Dr. Charles T.

Mohr, published in 1901 as a contribution from the United States National Herbarium.

In all of the reports on Alabama fungi mentioned before both parasitic and saprophytic forms on all hosts, regardless of their economic importance, have been included. In the present list only the fungi which occur on economic hosts have been considered. This list has been compiled from specimens and records of the various workers in Plant Pathology in the state since the publication of the paper by Underwood and Earle. In the main the record of fungi in this paper dates from October, 1920, for at that time the building housing all the records and specimens of fungi was destroyed by fire. This list is by no means complete but it is hoped that it may stimulate more interest in the subject so that all who can will add to our information.

Because there is no general agreement with regard to the names of fungus parasites those which are in most general use at present have been employed.

All economically important parasitic and non-parasitic diseases known to occur in the state have been listed. The parasitic diseases are given in alphabetical order according to their common name with the scientific name in parenthesis following. These are followed by the non-parasitic diseases and those for which no pathogen is known. These are indicated respectively by "Non-par" and "Undet."

The scientific and common names of the host included in this paper are those used in "Standardized Plant Names," as adopted by the American Joint Committee on Horticultural Nomenclature, 1923.

#### LIST OF ECONOMIC HOSTS WITH SOME OF THEIR DISEASES

Alfalfa (*Medicago sativa*): Crown Wart (*Urophlyctis Alfalsae*); Wilt (*Aplanobacter insidiosum*).

Alnus (*Alnus incana*): Catkin deformation (*Taphrina Robinsoniana*).

Apple (*Malus sylvestris*): Bitter rot (*Glomerella cingulata*); Blight (*Bacillus amylovorus*); Black rot (*Physalospora Cydoniae*); Black root-rot (*Xylaria* sp.); Blotch (*Phyllosticta solitaria*); Hypochnoze (*Corticium Stevensii*); Rust (*Gymnosporangium globosum*); Rust (*Gymnosporangium Juniperi-virginiana*); Scab (*Venturia inaequalis*); Septobasidium canker (*Seplobasidium pedicellatum*); Sooty blotch (*Gloeodes pomigena*).

Aster (*Callistephus chinensis*): Wilt (*Fusarium conglutinans Callistephi*).

Avocado (*Persea americana*): Leaf spot (*Phyllosticta micropunctata*).

- Azalea (*Rhododendron* sp.): Gall (*Exobasidium Vaccinii*).  
 Bean (*Phaseolus vulgaris*): Anthracnose (*Colletotrichum Lindemuthianum*);  
 Bacterial blight (*Bacterium Phaseoli*); Dry root-rot (*Fusarium Martii*  
*Phaseoli*); Leaf spot (*Cercospora cruenta*); Rust (*Uromyces appendiculatus*);  
 Southern blight (*Sclerotium Rolfsii*); Stem rot (*Corticium vagum*);  
 Mosaic (Undet.).  
 Bean, Scarlet Runner (*Phaseolus coccineus*): Leaf spot (*Cercospora cruenta*);  
 Rust (*Uromyces appendiculatus*).  
 Beech, Blue (*Carpinus caroliniana*): Powdery mildew (*Phyllactinia corylea*).  
 Bignonia (*Bignonia capreolata*): Leaf spot (*Cercospora capreolata*).  
 Blackberry (*Rubus* sp.): Rust (*Gymnoconia interstitialis*).  
 Cabbage (*Brassica oleracea*): Black leg (*Phoma lingam*); Black mold (*Alternaria Brassicae*); Black rot (*Pseudomonas campestris*); Downy mildew (*Peronospora parasitica*); Southern blight (*Sclerotium Rolfsii*); Yellows (*Fusarium conglutinans*).  
 Camphor (*Cinnamomum Camphora*): Anthracnose (*Gloeosporium Camphorae*).  
 Cantaloupe (*Cucumis Melo*): Downy mildew (*Peronoplasmodara cubensis*);  
 Leaf blight (*Macrosporium cucumerinum*).  
 Cedar, Red (*Juniper virginiana*): Cedar blight (*Phomopsis juniperovora*);  
 Rust (*Gymnosporangium bermudianum*).  
 Chrysanthemum (*Chrysanthemum* sp.): Leaf spot (*Cylindrosporium Chrysanthemi*); Wilt (*Fusarium* sp.).  
 Citrus (*Citrus* sp.): Anthracnose stain (*Colletotrichum gloeosporioides*); Canker  
 (*Bacterium Citri*); Melanose (*Phomopsis Citri*); Scab (*Cladosporium Citri*); *Septobasidium* canker (*Septobasidium pedicillatum*).  
 Clematis (*Clematis* sp.): Leaf spot (*Cylindrosporium Clematidis*).  
 Clover, Bur (*Medicago Medicaginis*): Leaf spot (*Cercospora Medicaginis*).  
 Clover, Red (*Trifolium pratense*): Anthracnose (*Colletotrichum Trifolii*); Powdery mildew (*Erysiphe Polygoni*).  
 Collard (*Brassica oleracea acephala*): Stem rot (*Sclerotium Rolfsii*); Wilt (*Fusarium conglutinans*).  
 Corn (*Zea Mays*): Brown spot (*Physoderma Zeae-maydis*); Root-rot (*Fusarium* sp.); Smut (*Ustilago Zeae*).  
 Cotton (*Gossypium hirsutum*): Angular leaf-spot (*Pseudomonas malvacearum*);  
 Anthracnose (*Glomerella Gossypii*); Ascochyta blight (*Ascochyta Gossypii*); Wilt (*Fusarium vasinfectum*).  
 Cowpea (*Vigna sinensis*): Anthracnose (*Colletotrichum Lindemuthianum*); Mildew (*Erysiphe Polygoni*); Rust (*Uromyces Vignae*); Scab (*Cladosporium Vignae*); Stem rot (*Corticium vagum*); Mosaic (Undet.).  
 Crape myrtle (*Lagerstroemia australiana*): Powdery mildew (*Uncinula australiana*).  
 Cucumber (*Cucumis sativus*): Anthracnose (*Colletotrichum lagenarium*);  
 Downy mildew (*Peronoplasmodara cubensis*); Wilt (*Bacillus tracheiphilus*).  
 Dewberry (*Rubus* sp.): Anthracnose (*Plectodiscella veneta*); Double blossom  
 (*Fusisporium Rubi*); Cane blight (*Leptosphaeria Coniothyrium*).  
 Dogwood (*Cornus* sp.): Leaf spot (*Cercospora cornicola*).  
 Eggplant (*Solanum Melongena*): Blight (*Phomopsis vexans*).  
 Elm (*Ulmus* sp.): Leaf spot (*Gnomonia ulmea*); Mildew (*Uncinula* sp.).

- Euonymus** (*Euonymus* sp.): Anthracnose (*Colletotrichum griseum*); Leaf spot (*Exosporium concentricum*).
- Fig** (*Ficus Carica*): Anthracnose (*Colletotrichum Carica*); Leaf spot (*Cercospora bolleana*); Rust (*Physopella Fici*).
- Geranium** (*Pelargonium* sp.): Bacterial leaf-spot (*Bacterium Erodii*).
- Grape** (*Vitis* sp.): Black rot (*Guignardia Bidwellii*).
- Hibiscus** (*Hibiscus syriacus*): Rust (*Kuehneola malvicola*).
- Hickory** (*Hicoria* spp.): Anthracnose (*Gnomonia Caryae*).
- Holly** (*Ilex* spp.): Tar spot (*Rhytisma ilicincola*).
- Iris** (*Iris* spp.): Leaf spot (*Heterosporium gracile*).
- Japonica** (*Camellia japonica*): Leaf spot (*Phyllosticta camelliaca*).
- Larkspur** (*Delphinium* spp.): Stem rot (*Sclerotium Rolfsii*).
- Laurel, Mountain** (*Kalmia latifolia*): Leaf spot (*Phyllosticta kalmicola*).
- Lettuce** (*Lactuca sativa*): Drop (*Sclerotinia sclerotiorum*).
- Lilac** (*Syringa vulgaris*): Leaf spot (*Cercospora Lilacis*).
- Magnolia** (*Magnolia* spp.): Leaf spot (*Phyllosticta Cookei*).
- Maple** (*Acer* sp.): Leaf spot (*Phyllosticta acericola*); Wilt (*Verticillium* sp.).
- Mulberry** (*Morus* sp.): Popcorn disease (*Sclerotinia carunculooides*).
- Oak** (*Quercus nigra*): Leaf blister (*Taphrina coerulescens*); Leaf spot (*Marsonia Martini*).
- Oats** (*Avena sativa*): Crown rust (*Puccinia coronata*); Loose smut (*Ustilago Avenae*).
- Okra** (*Hibiscus esculentus*): Wilt (*Fusarium vasinfectum*).
- Oleander** (*Nerium Oleander*): Leaf spot (*Macrosporium Nerii*).
- Onion** (*Allium cepa*): Neck rot (*Botrytis Allii*); Soft rot (*Bacillus carotovorus*).
- Palm** (*Phoenix* spp.): False smut (*Graphiola Phoenicis*).
- Peach** (*Amygdalus persica*): Brown rot (*Sclerotinia fructicola*); Crown gall (*Bacterium tumefaciens*); Curl (*Taphrina deformans*); Scab (*Cladosporium carpophilum*); Shot hole (*Bacterium Pruni*); Rosette (Undet.).
- Peanut** (*Arachis hypogaea*): Leaf spot (*Cercospora personata*).
- Pear** (*Pyrus communis*): Bitter rot (*Glomerella cingulata*); Blight (*Bacillus amylovorus*); Brown rot (*Sclerotinia fructicola*); Flyspeck (*Leptothyrium carpophilum*); Fruit rot (*Phytophthora cactorum*); Leaf spot (*Phyllosticta pyrina*); Leaf spot (*Pestalozzia Guepini*); Scab (*Venturia pyrina*); Septobasidium canker (*Septobasidium retiforme*).
- Pecan** (*Hicoria pecan*): Anthracnose (*Glomerella cingulata*); Brown leaf spot (*Cercospora fusca*); Crown gall (*Bacterium tumefaciens*); Myriangium disease (*Myriangium tuberculans*); Nursery blight (*Phyllosticta Caryae*); Powdery mildew (*Microsphaera Alni*); Scab (*Cladosporium effusum*); Septobasidium canker (*Septobasidium retiforme*); Rosette (Undet.).
- Peony** (*Paeonia* sp.): Blight (*Botrytis Paeoniae*); Leaf mold (*Cladosporium Paeoniae*).
- Pepper, Bell** (*Capsicum annum*): Bacterial wilt (*Bacterium solanacearum*); Rot (*Phoma destructiva*); Southern blight (*Sclerotium Rolfsii*); Blossom end-rot (Non-par.).
- Persimmon, Japanese** (*Diospyros kaki*): Leaf spot (*Cercospora fuliginosa*).
- Phlox** (*Phlox* spp.): Leaf spot (*Septoria divaricata*).
- Pine, Loblolly** (*Pinus taeda*): Rust (*Coleosporium delicatulum*).
- Pine, Longleaf** (*Pinus palustris*): Rust (*Cronartium cerebrum*); Rust (*Coleosporium delicatulum*); Rust (*Coleosporium Ipomoeae*).

- Pine, Shortleaf (*Pinus echinata*): Rust (*Coleosporium delicatulum*); Rust (*Coleosporium inconspicuum*).
- Plum (*Prunus domestica*): Plum pockets (*Exoascus Pruni*).
- Plum, Wild (*Prunus americana*): Black knot (*Dibotryon morbosum*); Brown rot (*Sclerotinia fructicola*); Hypertrophy (*Exoascus mirabilis*).
- Potato (*Solanum tuberosum*): Black leg (*Bacillus phytophthora*); Early blight (*Alternaria Solani*); Late blight (*Phytophthora infestans*); Scab (*Actinomyces scabies*); Scab, Powdery (*Spongopora subterranea*); Southern blight (*Sclerotium Roflsii*); Stem rot (*Corticium vagum*); Wilt (*Fusarium oxysporum*); Black heart (Non-par.); Mosaic (Undet.).
- Princess tree (*Paulownia tomentosa*): Leaf spot (*Phyllosticta Paulowniae*).
- Privet (*Ligustrum* sp.): Leaf spot (*Cercospora Ligustrii*).
- Raspberry (*Rubus* sp.): Cane blight (*Leptosphaeria Coniothyrium*); Mosaic (Undet.).
- Rose (*Rosa* spp.): Anthracnose (*Gloeosporium Rosae*); Black spot (*Diplocarpon Rosae*); Brown canker (*Diaporthe umbrina*); Crown gall (*Bacterium tumefaciens*); Mildew (*Sphaerotheca pannosa*).
- Rye (*Secale cereale*): Anthracnose (*Colletotrichum graminicola*).
- Snapdragon (*Antirrhinum Majus*): Rust (*Puccinia Antirrhini*).
- Sorghum (*Holcus sorghum*): Loose kernel-smut (*Sphacelotheca cruenta*): Rust (*Puccinia purpurea*).
- Soybean (*Soja Max*): Bacterial blight (*Bacterium Sojae*); Leaf spot (*Cercospora cruenta*); Southern blight (*Sclerotium Roflsii*); Mosaic (Undet.).
- Squash (*Cucurbita maxima*): Southern blight (*Sclerotium Roflsii*).
- Strawberry (*Fragaria* sp.): Leaf spot (*Mycosphaerella Fragariae*).
- Sugar cane (*Saccharum officinarum*): Eye leaf spot (*Helminthosporium Sacchari*).
- Sweet pea (*Lathyrus odoratus*): Anthracnose (*Colletotrichum Pisi*); Root rot (*Rhizoctonia Solani*).
- Sweet potato (*Ipomoea Batatas*): Black rot (*Ceratostomella fimbriata*); Charcoal rot (*Sclerotium bataticola*); Leaf spot (*Phyllosticta Batatas*); Rust (*Coleosporium Ipomoeae*); Stem rot (*Fusarium Batatas* and *Fusarium hyperoxysporum*); Mosaic (Undet.).
- Sweet William (*Dianthus barbatus*): Rust (*Puccinia Arenariae*).
- Tomato (*Lycopersicum esculentum*): Leaf spot (*Septoria Lycopersici*); Nailhead spot (*Macrosporium Tomato*); Southern blight (*Sclerotium Roflsii*); Stem rot (*Corticium vagum*); Wilt (*Fusarium Lycopersici*); Blossom end-rot (Non-par.).
- Turnip (*Brassica Rapa*): Black rot (*Bacterium campestre*); Leaf spot (*Colletotrichum Higginsianum*); Leaf spot (*Macrosporium herculeum*); Soft rot (*Bacillus carotovorus*); White rust (*Albugo candida*).
- Velvet bean (*Stizolobium* sp.): Leaf spot (*Phyllosticta Mucunae*); Stem rot (*Corticium vagum*).
- Violet (*Viola odorata*): Southern blight (*Sclerotium Roflsii*).
- Watermelon (*Citrullus vulgaris*): Downy mildew (*Peronoplasmopara cubensis*); Southern blight (*Sclerotium Roflsii*); Wilt (*Fusarium niveum*).
- Wheat (*Triticum aestivum*): Anthracnose (*Colletotrichum graminicola*); Glume blotch (*Septoria nodorum*); Leaf rust (*Puccinia triticina*); Loose smut (*Ustilago Tritici*); Stem rust (*Puccinia graminis*).

## NOTES AND BRIEF ARTICLES

Through some careless slip there has been a duplication of numbers in the plates for volume 22 of *Mycologia*. The number 13 has been repeated. Also the plates for the November-December issue should have been numbered 32-35 instead of 22-25. The volume, therefore, contains 36 plates instead of 25, as is indicated by the numbers. It is unfortunate that this error was not detected in time to prevent it.

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The Mycological Department of The New York Botanical Garden has recently mounted and filed in book form nearly 10,000 specimens of fungous exsiccati. Most of these are duplicates of specimens already distributed through the collection. However, as it is often difficult to locate these specimens by number it is very convenient to have duplicate sets filed in serial order. It is hoped that eventually all published sets of exsiccati may be so arranged.

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### FITZPATRICK'S PHYCOMYCETES<sup>1</sup>

As the amount of work on the fungi increases year by year, mycologists and plant pathologists find themselves more and more in need of adequate surveys of the main fungous groups. Realizing that the situation now demands an intensive treatment of each group, Dr. Fitzpatrick has started filling the long felt need by this volume on the Phycomycetes, a class of fungi which merits a more extended discussion than was possible in the helpful but necessarily restricted chapters in recent volumes by Gäumann, and by Gwynne-Vaughan and Barnes, on the fungi as a whole. The wisdom of Dr. Fitzpatrick's method of procedure is shown by the amount of material which he has presented in this volume and it is to be hoped that now he will go on and take up the other groups in like manner.

The form of the book accords with that adopted for others of

<sup>1</sup> Fitzpatrick, H. M. *The Lower Fungi—Phycomycetes*. 331 pages, illustrated. McGraw-Hill Book Company, Inc. New York, N. Y., 1930. Price \$4.00.

the recent McGraw-Hill publications in botanical sciences. The volume is a neat, compact one, firmly bound, attractively covered and lettered, printed in a clear, easily read type on paper not too heavy, yet thick and smooth enough to take illustrations well. And the book of 331 pages, abundantly illustrated, is issued at a price not beyond the means of the college student.

Following the brief preface in which the purpose of the volume is given, Chapter I, an introduction of 18 pages, discusses the nature, development, characteristics and relationships of the several groups of Thallophytes as a background for the specific study that follows, and Chapter II, comprising 20 pages, covers such general points as the origin of the Phycomycetes, the nature of the thallus and of the asexual and sexual methods of reproduction, the classification of the group, its basis, and the orders recognized in this classification, while at the end of the book a final Chapter XI in 12 pages discusses the phycomycetous affinities of the Hemiascomycetes, taking up the possible significance of various forms which have been stressed as important in this interesting but much disputed question.

Chapters III to X, inclusive, comprise discussions respectively of the Chytridiales, 11 pages; the Ancylistales, 11 pages; the Blastocladiales, 6 pages; the Monoblepharidales, 7 pages; the Saprolegniales, 34 pages; the Peronosporales, 38 pages; the Mucorales, 41 pages; and the Entomophthorales, 17 pages. In each of these, keys to the families and genera are given, the salient characteristics of the order and of its several families are presented, and the structure, development, life history, importance, and relationship of the various genera are taken up. As can be seen, some orders are emphasized more than others and are given a much more thorough discussion because of their unusual scientific interest or their outstanding economic importance.

One very useful feature of the book is the provision of a separate bibliography at the close of each chapter, a procedure which makes it easier for the student to gain a knowledge of the literature dealing with any group, facilitates his looking up material for additional reading and gives him a readily accessible source list for consultation.

The index at the end of the book is satisfactorily detailed and

extensive, adequately meeting the needs of a reference book of this type.

The author takes up the terms which are used in the text and explains them when they are first encountered, thus avoiding a formal glossary and presenting the terms and their definitions in the context in which they belong.

The illustrations are numerous, 112 figures, mostly zinc cuts photographically reproduced from line drawings, a few half-tones from previously published plates, and several illustrations presented in this book for the first time.

In a book review such as this, when the purpose, the form, and the scope of the volume have been covered and a general survey of the content has been given, it seems an established custom to turn next to points of criticism.

If this procedure is to be followed, attention should be called to the fact that the discussion of the "zygospores" and their method of formation in *Dispira americana*, as attributed to Dr. Thaxter on page 271, in reality describes and figures the "sikyospores" of the parasitic, sporangial, mucoraceous genus *Parasitella*. The perpetuation of this error is unfortunate, the more so since, in 1903, Blakeslee in his "Sexual Reproduction in the Mucorineae," page 245, had already explained, "Professor Thaxter informs me that the zygospore-like bodies found by him in connection with his cultures of *Dispira* were probably accidentally associated with it, and that they are doubtless referable to the genus *Parasitella* recently published by Bainier ('03)," while Burgeff in his paper of 1924 (included by Fitzpatrick in the bibliography following this chapter) unmistakably describes and figures these "sikyosporen" of *Parasitella*. Moreover, in accepting Lendner's relegation of *Parasitella simplex* Bainier 1903 to *Mucor parasiticus* Bainier 1884, Fitzpatrick must have seen Lendner's Figure 24 of the "tuberousites" or "renflements" of the parasite, recognizable as the so-called "zygospores" of *Dispira* even though Lendner's portrayal had the crudeness of some primitive caveman, or of the most astigmatic of moderns. Incidentally, the wisdom of accepting Lendner's relegation might be questioned, as Bainier's later judgment probably should supersede

his earlier one and Burgeff, Blakeslee and others who recently have worked with *Parasitella* retain it as a distinct genus.

Also it is unfortunate that in the subgenus *Sphaerosporangium* (p. 198) there are included a number of species of *Pythium* characterized by lobose or filamentous sporangia wholly distinct from the true *Sphaerosporangium* type.

Then, too, it hardly seems just that the Endogoneae, recognized as a distinct family in the most recent revision by R. Thaxter, should be relegated to the ignominious position of an appendage to the Mortierellaceae, scantly noted in connection with *Mortierella*, and omitted from the key to the families.

Moreover, it is perhaps regrettable, when more than half a page is given to the theoretical explanation of the mechanism of *Pilobolus'* orientation to light, that there is no mention of the significant experiments by Jolivette and others on this phenomenon, or by Blau and others on the related problem of the influence of light on the growth and orientation of *Phycomyces*.

Likewise it is unfortunate that in discussing the amphigynous type of antheridia in *Phytophthora* (p. 203) no credit is given to Dastur's paper of May 1913 which shares with Pethybridge's paper of March 1913 the distinction of calling attention to the extraordinary method of forming these bodies.

As several of the excellent figures from it aid in illustrating the Peronosporales, the lack of reference to Schwartz's "Parasitic Fungi of New Jersey" must have been merely an oversight. The significant papers by Kolkwitz (Ber. d. D. Bot. Ges. 1901-1903) on artificial culture of *Leptomitus* might well be included in the bibliography, while mention of Morini's *Phycomyces pirottianus* calls for reference to his "Note Micologiche" in Malpighia 10, 1896.

The genus *Coenomyces* might well be mentioned, not because of the possible phylogenetic significance stressed by Deckenbach, who described it (Flora 1903), but because of its interesting habitat, distribution and parasitism.

In the brief discussions of Scherffel's work, lack of reference to some of the extraordinary points brought out arouses a suspicion that Fitzpatrick, like the present reviewer, has found those papers almost Brefeldianly hard reading.

The illustrations, on the whole, are excellent. Some, however, have suffered at the hands of the engraver, either through carelessness in cutting out the figures on the block, as in Figs. 51 and 65a, or through blotchy rendering of lines and stippling originally distinct, as in Figs. 52, 63, and 76. Also, some fall far short of the excellence of the originals from which they have been taken; for example, compare Figs. 55a-c of *Aplanes* and Fig. 59 of *Pythiopsis* with the lithographs of de Bary's Taf. 9 in the Botanische Zeitung for 1888, or Figs. 61e, f, of *Achlya* with the wood cuts of DeBary's Fig. 70 in the 1887 "Comparative Morphology," or Fig. 70 of *Pythiogeton* with Von Minden's Plate 7 in his "Submerser Phycomyceten" of 1916. In one or two cases errors have crept in during preparation so that the figures are actually incorrect. For example, in Fig. 60 of *Saprolegnia*, the proliferated sporangia at *a* show inaccuracies in the size and orientation of the escape pores and in the position of the basal wall, features correctly represented in Plate 9, Fig. 1, of Coker, 1923; and in *b* through line-shading the walls of the oöspores and oögonium, the accurate outlines of Coker's Plate 11, Fig. 1, have been lost; while in *e* the wall thickness of the non-motile spores and the shape and ciliation of the zoospore have lost the accuracy of Marshall Ward's Plate 28 of 1883. Moreover, in *c* of this same Fig. 60, the obvious error in thickness of the sporangium wall, inherited through Atkinson's figure of 1909 from the original ancestral Fig. 134 of Atkinson's "Elementary Botany" of 1898, regrettably is perpetuated. Also, Figs. 78 to 80, although very cleanly reproduced, would perhaps be more effective if the tops of the conidiophores were up instead of down, as in their present position they are more difficult to apprehend even though one realizes that in many instances it is in this very position that they grow from the leaves.

All of us, alas, have found that rarely do we publish even a short paper without at least one typographical error; in this book there are many still to be corrected. Pages such as 70 or 76 probably lead in number of these, but page 240 with "gaem-tangia" and pages 247 and 248 with "Syzgites" deserve recognition for novelty at least. Attention should be called to the correctness of Piptocephalidaceae rather than Piptocephalaceae, Brefeld's work on *Conidiobolus utriculosus* (not *utriculosis*) was

in *Mycologische Untersuchungen* 6, not 4, Sparrow's note on "Rotifer Capturing Phycomycetes" was in 1929, not 1919, and the date and reference for the establishing of the genus *Sclerospora* should be *Hedwigia* 18: 87, 1879.

Throughout the book "corresponds with" and "corresponds to" are used with large-hearted impartiality; under Fig. 54, "Magnification of *a* and *e* is the same; that of others same but higher" leaves the reader somewhat bewildered; "monoplanetic as regards form" for *Pythiopsis* is rather obscure; while on page 198 the sentence, "The fungus *Rheosporangium aphanidermatum*, cause of a disease of radish known as black-root and damping off of beet seedlings," as it now stands leaves one feeling that there is little hope for the radish thus doubly afflicted.

The term aboospore, which apparently is used here for the first time, seems somewhat ill chosen, sure to cause confusion when received by ear during hurried lectures, and to the eye appearing as if it had something to do with a cry of derision. Moreover, the word planogamic is of doubtful etymology, planogametic perhaps being preferable.

If, in the foregoing criticism, I have seemed overzealous in pointing out errors, imperfections and omissions, let it be remembered I am convinced that the value of this book soon will necessitate a second edition, and toward such a revision these suggestions will be helpful. Also in critically scrutinizing the book I have learned much; for example, from looking up the original description of *Papulaspora* I have learned that we have been spelling this name incorrectly in this laboratory for years. Moreover, I am aware that this, Dr. Fitzpatrick's first book, represents a total of exactly one book more than I (or several other critics) have written as yet. The book is one of substance and worth. We are finding it a useful and valuable compendium of material that has not been made accessible hitherto. Furthermore it is to be commended for the able and impartial presentation of such controversial questions as the possible phylogeny of the Phycomycetes, and the possible relationships between the Ascomycetes and Phycomycetes. Very helpful also are the clarifying discussions of the disputed distinctions between *Pythium* and *Phytophthora* and between conidia and sporangia, in their

relation to the influence of external conditions on methods of discharge or germination. It seems certain that this volume will stimulate researches into the many significant problems the Phycomycetes present and it is to be hoped that Fitzpatrick may be induced to take up the higher groups of fungi in the same way.

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#### ANNOUNCEMENT

Doctor Jacob E. Lange, well known Danish student of the mushrooms, will arrive in New York the middle of August for several weeks of collecting in the northeastern United States and eastern Canada. He wishes to study especially the parallelism and identity of American and European species of Agaricaceae. A definite itinerary has been arranged. Inquiries regarding its details may be directed to Doctor C. W. Dodge at Pawlet, Vt.

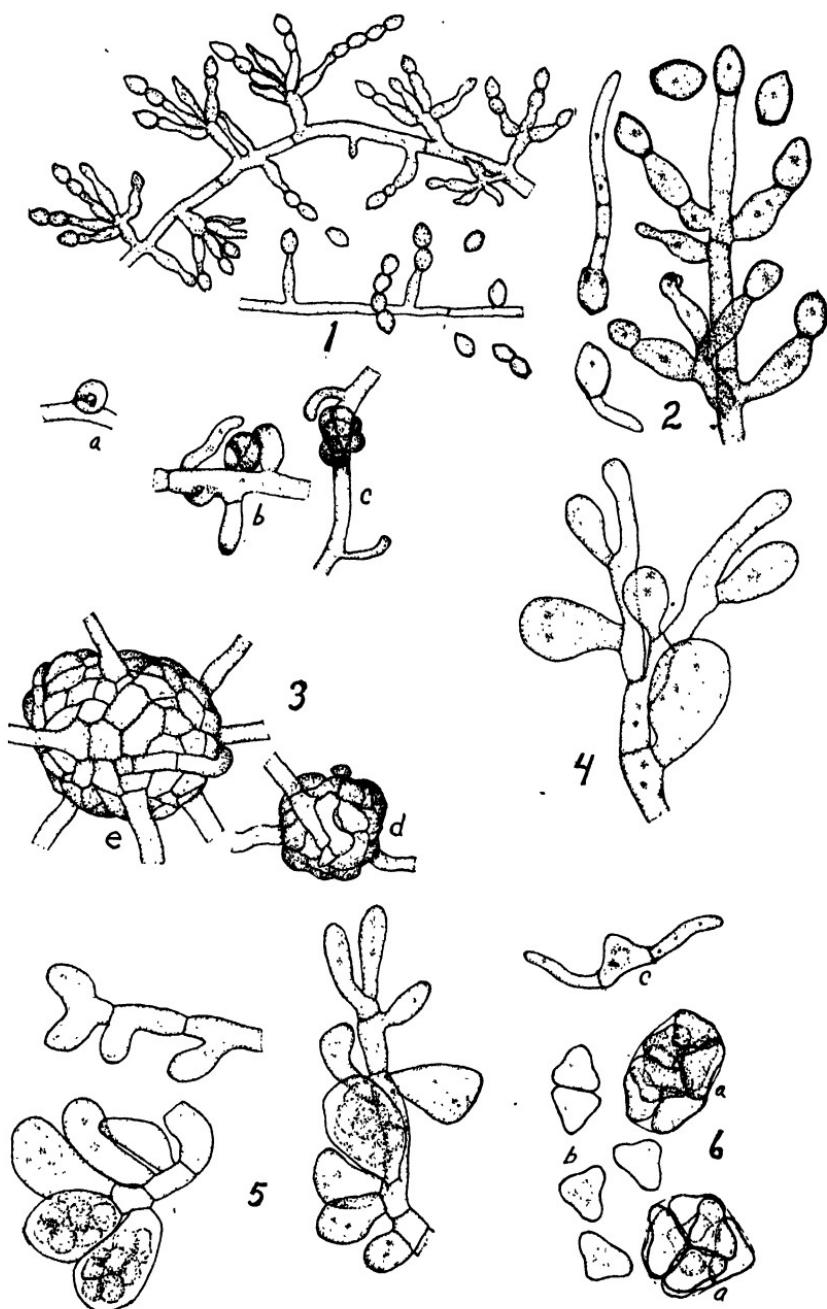
From August 28 to September 2 inclusive Doctor Lange will be at Ithaca, New York. The region about Ithaca is especially interesting to him because Atkinson published over a period of years on locally collected materials. Fungus forays will be made daily to nearby points of interest in the effort to see a large number of species.

In order that the conceptions of species as held by Peck, Atkinson, Kauffman and other older American workers in the group may be clearly understood, it is imperative that Doctor Lange be enabled to exchange ideas in the field with their students. To this end American mycologists, especially those interested in mushrooms, are urged to come to Ithaca and co-operate in making these forays a success. Students with only a minor interest in the Agaricaceae will also be welcomed, and the forays will be arranged in such a manner that collecting in other groups will be fruitful. Incidentally, the Atkinson herbarium has been put in good order in recent years, and is now available for consultation in the new Plant Science Building at Cornell University.

Those who plan to attend the Ithaca forays are asked to notify the undersigned at as early a date as possible. Arrangements will be made for lodging, meals, and transportation at reasonable rates. Information concerning these items or other features of the plans for the forays will be gladly given.

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MICROASCUS TRIGONOSPORUS

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## THE ASCOCARPIC STAGE OF SPECIES OF SCOPULARIOPSIS

C. W. EMMONS AND B. O. DODGE

(WITH PLATES 25-28)

### INTRODUCTION

The asexual fructifications of ascomycetes are as often as not misleading when it comes to determining relationships. *Monilia* may be the conidial stage of certain species of *Sclerotinia*, a discomycete, on the one hand, or it may be connected with species of *Neurospora*, a pyrenomycete, on the other. *Botrytis* may be the asexual stage of certain other species of *Sclerotinia* in one case, but be connected with a *Lachnea*, quite a different type of discomycete, in another case. Much of our knowledge of the morphology of the ascocarp of *Aspergillus* and *Penicillium* dates back to the time of de Bary, Zukal and Brefeld, and to the cytological work of Fraser and Chambers and of Dale. The group Plectascales may be a heterogeneous one including forms of doubtful relationship. Should *Thielavia* as represented by *T. basicola* and *T. terricola* be included along with *Aspergillus* in this group? Neither species of *Thielavia* has a conidial stage and their carbonaceous ascocarps are quite unlike those of either *Aspergillus* or *Penicillium*. The disposition of the ascogenous hyphae and asci is such, however, as to suggest in a general way a relationship.

The writers have recently had under observation two species of ascomycetes which in the manner of the origin and development of the ascogenous hyphae and asci, taken in connection

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with the type of ascocarps and conidial stages, seem to furnish evidence bearing on this point, and indicate a relationship between such forms as *Penicillium*, *Scopulariopsis* (*Acaulium*), *Thielavia*, *Microascus*, and *Chaetomium*. Although the gap between the Plectascales and the Sphaeriales here is a very narrow one, the differences between the ascocarps of *Scopulariopsis* and *Chaetomium* are such, no doubt, as to warrant their separation.

Among the species of fungi isolated from roots of strawberries affected with the disease known as "dwarf" were two of particular interest. One was a species of *Thielavia*, referred to above and first described as *Coniothyrium terricola* by Gilman and Abbott (10). Emmons (8) has, however, proved that what were taken for pycnidia were really ascocarps. Unless one studies rather young material he is apt to fail to see the asci which deliquesce very quickly leaving the ascospores massed together within the cleistocarpous fruit body. In this condition the spores might easily be mistaken for pycnospores. The second species was noteworthy because of the way the little black fruit bodies discharged their coffee-brown spores in long contorted cirri. As not many ascomycetes discharge the spores from their perithecia in this way, one might think that here too the fruiting structures are pycnidia, especially as at the time when the spores are being discharged freely, asci are not readily distinguished.

Very similar to the latter was one of the fungi cultured by Kesten (13) from a superficial fungus infection occurring on the feet and legs of a Porto Rican. Three fungi were found in cultures from the lesions. On group study at the Mycology Laboratory these fungi were found to be *Epidermophyton inguinale*, *Geotrichum candidum*, and a species of *Scopulariopsis*. The *Epidermophyton* was probably the cause of the skin lesions. The other fungus cultures secured from this case very likely developed from spores which chanced to be present on the skin. The second species will not be considered further. The third species, by the character of its conidial fructifications was recognized as a species of *Scopulariopsis* some species of which cause disease. *S. cinerea* to be mentioned later has, for example, sometimes been found causing a disease of the nails. The fungus

had other very interesting characters as will be pointed out, and it was very kindly turned over to the authors for further study. These two fungi, the one isolated from strawberry roots, the other from a skin lesion form the subject of this paper.

#### RELATED PLECTASCALES

Two new species of fungi described by Zukal (22, 23) as growing on dung or other refuse were included by him in a new genus *Microascus*. *M. sordidus* is a very small reddish-brown pyrenomycete with a papillate ostiole which may sometimes develop a short neck as is shown in his figure, copied in Engler and Prantl, who place this genus among the Aspergillaceae. The ascospores are reddish-brown, elliptical, rounded at both ends, inequilateral and  $9-9.5 \times 5.5-6 \mu$ . Zukal says of one characteristic: "Die reifen Sporen werden bei diesere Species rankenförmig aus dem kurzen Halse hervorgepresst und bilden ausserhalb des Fruchtkörpers lange, röhliche kantige Schnüre." Zukal does not mention any conidial stage connected with the two species he described.

Massee and Salmon (18) in their studies of coprophilous fungi describe two species which they place in the genus *Microascus*. Their *M. variabilis* has ascospores very similar in shape to those of *M. sordidus*, but they are much smaller, being only about  $3-3.5 \mu$  long. No conidial stage is mentioned.

Bainier in 1907 (1) created a new genus, *Scopulariopsis*, with *Penicillium brevicaule* Sacc. as the type mainly on the basis of differences in the conidia and the form of conidiophore branching between this species and a true *Penicillium*. *Scopulariopsis* is discussed at some length by Thom (21) who is inclined to agree with Bainier, holding particularly that species showing a collar at the base of the conidium with germination through a basal germ pore, should not be included under *Penicillium* proper.

Sopp's monograph of the *Penicillium* group (20), 1912, was evidently published without knowledge of Bainier's work. With the group species, *P. brevicaule* also in mind, Sopp makes a new genus, *Acaulium*, which would have no particular standing were it not for the fact that three of his new species have very characteristic ascosporic stages. The very close resemblance of

these forms and the species of *Microascus* already mentioned raises a further doubt as to the validity of the generic name created by Sopp. Thom (21) discusses the seven species of *Acaulium* in his chapter on *Scopulariopsis* but still using Sopp's genus name. According to Sopp, *Acaulium albo-nigrescens* in culture shows a snow-white mycelium with long chains of oval or oblong white conidia. The ascocarp is a very small, black, spherical to pear-shaped structure with a distinct ostiole. The asci are spherical. The coffee-brown ascospores, when mature, are discharged in such abundance as to change the color of the old culture from black to brown. The spores are kidney-shaped, about  $6 \times 4 \mu$ , cemented together in clumps with a substance which hardens and is not readily soluble in water. The spores separate quickly, however, in a drop of potassium hydroxide or acetic acid.

The one new species of *Dactylomyces*, so named by Sopp because of the finger-like conidial heads, grows best at about 40° C. The perithecia of *D. thermophilus* are reddish-brown, globose, and about 1/2 mm. in diameter. The wall cells are very large and angular. The oval to globose asci deliquesce, and the small yellowish ascospores escape through a very fine ostiolar opening. Sopp thinks this species may possibly be the same as *Thermoascus aurantiacus*, described by Miehe (19). Both species are reported as growing best at high temperatures.

Emile-Weil and Gaudin (7) report finding in several cases of infected great toe a new species of fungus, *Scopulariopsis cinerea*. Small black globose perithecia developed in their cultures. The oval asci deliquesced early, leaving the spores, which were brown, plano-convex,  $6-7 \times 3-3.5 \mu$ , free in the perithecial cavity. No ostiole was mentioned, but if it were no more conspicuous than the ostiole of *Dactylomyces* figured by Sopp, it might escape notice.

Lechmere's (14) *Peristomium desmosporum*, described in 1913, also recalls Zukal's *Microascus* by the way the reddish-brown ascospores, united by some cementing substance, are discharged in long cirrhi, and by the general form of the peritheciun, asci and spores. The conidial stage, which Lechmere obtained only from a particular strain, is described as of the verticillate

type. His figures are not unlike those of certain species of *Scopulariopsis* with reduced spore chains. It was, however, the manner in which the ascospores were discharged in long cirri that attracted his attention and suggested the specific name *desmosporum*.

Thom also points out that *Scopulariopsis candida* (Pers.) Loubière (*Monilia candida* Pers.?), connected by Loubière (15) with an ascomycetous stage described as a new genus and species, *Nephrospora Mangini*, might well be the same fungus described previously by Sopp as *Acaulium albo-nigrescens*. A recent comparison of cultures of these species obtained from the Baarn Culture Laboratory indicates, however, that they are not the same.

Besides four species of *Microascus*,<sup>1</sup> we have, then, *Acaulium albo-nigrescens*, *Peristomium desmosporum*, *Scopulariopsis cinerea*, *Nephrospora Mangini*, and the two forms studied by the writers, all of which are clearly so closely related as to warrant their inclusion in one genus, were it not for the conidial stage, now present, now absent. The writers are not particularly concerned to attempt to straighten out such a taxonomic tangle, even if all the facts relating to the questions of morphology were known. The first species to be described here has a conidial stage which Dr. Charles Thom has pronounced, after examination, to be a good *Scopulariopsis*. Without this conidial stage the fungus would be classed in the genus *Microascus*, or in one separated from it principally by the shape of the ascospores. We prefer to adopt this old genus name, *Microascus*, instead of one of the later names *Acaulium*, *Peristomium* or *Nephrospora*, and leave *Scopulariopsis* as the form genus under which the conidial stage can be described. The following is a formal description of the fungus as it grows in culture. Its usual substratum in nature is not known.

#### *Microascus trigonosporus* sp. nov.

Conidial form (*Scopulariopsis trigonospora* nom. nov.). Cultures on corn-meal agar sparse, gray becoming light-brown with the formation of conidia and finally dotted more or less closely

<sup>1</sup> A paper by M. Curzi on "Una nuova specie di *Microascus*" (Boll. R. Staz. Pat. Veg. 10: 302-310. 1931) has just come to hand too late for comment in the text. The species he describes is very similar to our *M. trigonosporus*, but the two species are distinct.

with the black ascocarps. Mycelium composed of branching, septate hyphae  $1.5\text{--}3 \mu$  in diameter, homothallic. Conidiophores lacking, or simple and unbranched, bearing at the tip a chain of spores, or bearing at the tip a single verticil of sterigmata, or variously branched and bearing more or less distinct whorls of sterigmata. Sterigmata  $5\text{--}7 \mu$  long, usually inequilateral, with the diameter greatest near the middle where it becomes  $2.5\text{--}3 \mu$ , constricted at the base and tapering toward the apex. Conidia brown, oval to lemon-shaped, with a collar at the base,  $2.7\text{--}3.2 \times 4.3\text{--}5.5 \mu$ , germinating from the side or rarely from the base. (PLATE 25, FIGS. 1, 2.)

Perithecial stage: Ascogonium coiled, antheridium not observed. Ascocarp globular with papillate ostiole, often with a pronounced beak,  $125\text{--}235 \mu$  in diameter on corn-meal agar, very early becoming black, the outer five to eight layers of cells heavily carbonized, the ascogenous hyphae growing downward from the ascogonium, which is then located well up under the ostiole. Asci oval,  $7\text{--}9 \times 8\text{--}11 \mu$  on corn-meal agar, irregularly distributed and in general oriented toward the periphery of the ascocarp and away from the ascogonium, eight-spored, deliquescent within the ascocarp. Ascospores triangular, but often thickened on one side so that some are almost four-cornered,  $3\text{--}3.8 \times 4.5\text{--}5.5 \mu$ , discharged from the drying ascocarp as a long, light reddish-brown cirrus  $25\text{--}45 \mu$  in diameter and reaching a length of 4 or 5 mm. (PLATE 25, FIGS. 3-6; PLATES 26, 27.)

Type locality, Porto Rico.

Type specimen deposited in the herbarium of The New York Botanical Garden.

The fungus was first isolated and grown on Sabouraud's maltose agar medium. On this medium it produces a luxuriant growth with an abundant gray aerial mycelium and numerous ascocarps. The colonies are restricted in extent and become wrinkled and raised above the surface of the agar. Within ten days or two weeks black ascocarps begin to develop in the aerial mycelium. As the culture grows older there is often a zone of ascocarps formed on the surface of the agar around the denser portion of the colony. These ascocarps may be quite closely

crowded together while there is scanty production of aerial hyphae in this region.

On corn-meal agar the growth is less luxuriant and there is no buckling of the surface. The diameter of the colony may be greater than it is on maltose. The color of the young colony is gray but it becomes light-brown with conidial production. When cultures are kept at 37° C. the mycelium becomes dark-olive. Fewer ascocarps are produced than on maltose agar and the temperature range for ascocarp production is narrower, the optimum seeming to lie between 28° and 30° C. Gelatin is liquefied but there is no apparent odor of arsene.

The conidial stage of *M. trigonosporus* is typical of *Scopulariopsis*. The conidiophore is sometimes simple and unbranched, arising directly from the mycelium and bearing chains of conidia. This structure may be very short or even wanting, in which case the conidium is borne directly upon the mycelium. This sometimes occurs on submerged hyphae. In other cases the conidiophore is more richly branched and may bear more or less perfectly developed verticillate whorls of sterigmata (PLATE 25, FIG. 1). The sterigmata are swollen at the middle portion, constricted at the base, and taper toward the apex. They are often asymmetrical, being more swollen on one side than on the other. The chain of spores is enveloped by a thin membrane, most easily seen under oil in fresh mounts in sodium hydroxide solution. The conidia are uninucleate, smooth, oval to lemon-shaped, usually inequilateral, and with a thickened basal collar. Germination takes place from the side of the spore, usually from the side which is bulged and sometimes from both sides. In old spores germination is sometimes through the base, as it is reported to occur commonly in other species of *Scopulariopsis*.

Monospore cultures of the fungus show it to be homothallic. At least six cultures were secured from single conidia and twenty from single ascospores. All cultures produced both conidia and ascospores. The perithecia are highly carbonized and brittle and produce an audible sound when crushed under a cover slip. The outer wall of the perithecium is composed of five to eight layers of carbonized, thick-walled, flattened cells. The inner wall is composed of four or five layers of larger thin-walled

cells, also flattened tangentially, and enclosing the sterile tissue and ascogenous hyphae.

Certain features in the development of the ascocarp are of considerable interest because of the information as to relationships which they may give. The ascocarp originates from a coiled ascogonium (PLATE 25, FIG. 3). In some cases hyphae which may have been antheridia were observed but in other cases such structures appeared to be absent. The ascogonium soon becomes enveloped by hyphae and the further development is shown by a study of sections. These show that the ascogonium is septate and the cells contain several nuclei (PLATE 26, FIGS. 1, 2). The young ascocarp at first is globular and then the ascogonium lies at its center. This relationship of its parts is maintained as the ascocarp increases in size by growth and cell division until it is 40-50  $\mu$  in diameter and the ascogonium is surrounded by about 8 or 10 cell layers. The cells in these layers are flattened tangentially and those of the outer layers are carbonized. The size of the young ascocarp is now increased by the elongation of these cells tangentially to the ascocarp. The space which such a type of growth would otherwise leave around the ascogonium is immediately filled by an inward growth of the cells which surround the ascogonium (PLATE 26, FIGS. 2-6). As space permits, they increase in length, until they become septate, slender, finger-like tapering hyphae growing inward from the inner perithecial wall. However those below the ascogonium develop much more rapidly and further than those beside or above it. As a result of this development the cell layers in a median section of the young ascocarp are concentric only toward the periphery. Inside these layers, which form the inner wall of the perithecium, there is a strong development of inward growing sterile hyphae from below, and a weaker development of inward growing hyphae from the sides and above. As a result of this differential growth the ascogonium comes to occupy a position above the center of the ascocarp instead of near the base as in most perithecia. At the same time, or a little later, there is evidence of ostiolar development. This is initiated by differential growth, which results, by tangential elongation of the inner perithecial cells, in an ostiolar cavity

schizogenetically formed (PLATE 27, FIGS. 2, 3). The elongation of these cells continues so that at maturity there is a papillate ostiole lined with short periphyses. In many cases a very pronounced beak forms (PLATE 27, FIG. 3).

Ascogenous hyphae have meanwhile grown out from the ascogonium and, because of the position of the latter up under the ostiole, most of this growth is downward although it occurs to some extent radially in all directions. These ascogenous hyphae branch freely but their radial orientation from the ascogonium remains at all times apparent. In this form croziers have not been observed. The asci appear to arise as side or terminal branches of the ascogenous hyphae. It may be possible that croziers sometimes occur, and in the closely related form to be discussed later structures which suggest them are sometimes present. The first asci form in the region near the location of the ascogone, that is, above the central cells of the ascocarp. The ripening of asci proceeds from this region peripherally, following the direction of growth of the ascogenous hyphae. The last asci to form are near the periphery toward the base of the ascocarp.

The orientation of the asci seems to depend entirely upon the direction of growth of the ascogenous hyphae and their mutual pressure. Consequently they are oriented in general toward the periphery of the ascocarp. This orientation is very definite in those which are at the extreme edge of the cavity next the perithecial wall. Although one is reminded of the peculiar position of the asci in catothecia and thyriothecia discussed by von Höhnel (11, 12), the organization of the ascocarp here is wholly different and there is no reason to suppose that the groups he referred to are related to *Microascus*.

The asci are oval and are eight spored. They remain intact for a considerable period after the spores appear to be mature, but eventually they deliquesce. They no doubt contribute to the cementing substance which holds the spores in the cirrus which is characteristic of this and related forms. The spores are yellowish-brown to fuliginous under the microscope. They appear to be triangular when examined. When rolled over in a liquid mount they are seen to be quite thick, and many are

sufficiently thickened at one point to form a fourth corner which is also visible upon focusing upward. The spore resembles in form and color that of *Bommerella trigonospora* as described by Marchal (16) but since the ascus of *B. trigonospora* is clavate and since, on other morphological grounds, that genus is presumably one of the Sordariaceae, the resemblance of the spores is no doubt only an accidental parallelism. *Bommerella* was separated from *Chaetomium* by its author largely on the basis of its triangular spores, but Chivers (5) includes it in *Chaetomium*. Students of the discomycetes usually consider species with spherical spores as generically different from those with elliptical spores. According to this line of reasoning it might seem that *Microascus trigonosporus* should not be included in the genus *Microascus*, the type of which, *M. longirostris*, has crescent-shaped spores. The evolution of either a triangular or a crescent-shaped spore from the kidney-shaped spore of *M. sordidus* would involve only a very slight further emphasis of the irregularity already initiated.

The spores of *M. trigonosporus* are set free within the ascocarp and as the culture dries (after two or three months at room temperature) the spores are slowly extruded as a cirrus. This cirrus development may require several weeks for completion and on the same agar slant one can find some ascocarps which still contain all their spores, some in which the spore mass is just beginning to emerge, and others in which spore discharge appears to be completed. The diameter of the cirrus is from 25–45  $\mu$  and it may reach a length of at least 4 mm. Its color is reddish-brown. When sowed upon agar or in a moist chamber the spores germinate by sending out a germ tube from one or more corners (PLATE 25, FIG. 2). The spores at maturity are uninucleate, but since colonies arising from single spores produce ascocarps the species is homothallic.

*Microascus trigonosporus* is of considerable interest because of the peculiar organization of its ascocarp and the type of its conidial stage. These are such as to suggest that it may form a connecting link between divergent groups. The conidial fructification resembles in a general way that of *Penicillium*. Thom (21) points out that modern students of the groups do

not consider *Penicillium* and *Scopulariopsis* to be closely related. A superficial examination of ascocarps of *Microascus trigonosporus* and of similar ascocarps described by Sopp under the genus *Acaulium* and a comparison of these with the type of ascocarp found in some species of *Penicillium* support this view. However, when one comes to examine sections of mature ascocarps of the two types one finds that the arrangement of the ascogenous hyphae and distribution of the asci are similar. When, on the other hand, *Microascus trigonosporus* is compared with a member of the Sphaeriales such as *Sordaria* the external resemblance is seen to be strong, and in certain species of *Chaetomium*, especially, the asci tend toward the oval shape and deliquesce early. However, the internal arrangement of sterile and fertile tissue is quite different because of the peculiar position of the ascogonium and the ascogenous hyphae in *Microascus*.

Apparently most species of *Chaetomium* do not discharge their ascospores forcibly. The asci deliquesce early so that the spores lie free in the perithecial cavity from which they ooze out through the ostiole and tend to accumulate in the form of a tall heap. *C. trigonosporum* (*Bommerella trigonospora*) and *C. quadrangulatum*, Chivers says, are the only species having angular spores which he includes in the genus *Chaetomium*. It should also be noted that in these two species the spores are discharged in long narrow cirri. *Microascus variabilis* (18) develops a few hairs from the neck portion of the perithecium. *Chaetomium pusillum* and *C. chartarum*, both of which Chivers places in an allied genus *Ascotricha*, have conidial stages. The conidia are at first hyaline and then olivaceous-brown. We thus have a number of forms which on various morphological grounds show relationships. In species of *Aspergillus*, *Penicillium*, *Thielavia* and *Microascus*, the ascogenous hyphae grow out in all directions from the more or less centrally placed ascogonium. So far as known, the ascogenous elements of *Sordaria* and *Chaetomium* arise from the ascogonium placed well below in the perithecium, providing a fairly definite hymenial layer characterized by rather long asci.

The second species with which this paper deals is very much like *Microascus trigonosporus* in the origin and development of

the ascocarp. Although we have not succeeded in obtaining conidia, the fungus otherwise resembles in some particulars *Acaulium albo-nigrescens* (20). For reasons which have been stated the fungus is described below as a new species of *Microascus*.

***Microascus intermedius* sp. nov.**

Perithecia black, pear-shaped, spherical or somewhat flattened,  $75-150\mu$  in diameter; ostiolar portion more or less papillate in mature fruit bodies; ascogenous hyphae growing outward and principally downward from an ascogonium usually placed somewhat above the center of the perithecium; asci oval to spherical,  $7.5-10 \times 10-11\mu$ , walls deliquescent early, leaving the spores free in the central cavity, eight-spored; individual ascospores very pale yellowish-brown, inequilateral or concave convex,  $3.5-5.5\mu$ , discharged in long contorted coffee-brown cirri. (PLATE 28.)

On decaying strawberry roots.

Type locality, Chadbourn, N. C.

Type specimen culture deposited in the Herbarium of The New York Botanical Garden.

On ordinary corn-meal agar the mycelium is snow-white with very little aerial growth. It grows very slowly here as on all other media. Minute black specks, sclerotia, can just be made out with a hand lens. Olive-black, somewhat flattened perithecia begin to appear about the second week and become scattered rather thickly about the point of inoculation. The fungus was proved to be homothallic by the fact that single spore cultures always produce perithecia on this agar medium, either at room temperature or when incubated at  $25-27^\circ\text{ C}$ .

On "Difco" corn-meal agar the mycelium is at first snow-white, but soon becomes dark-olivaceous or brownish, assuming a dendritic form of growth. Many little dark-colored sclerotia which can be seen without a lens are formed. Such cultures have been held at room temperatures, others have been incubated, but none has produced perithecia on this kind of agar. Transfers from these cultures to ordinary corn-meal agar begin to produce ascocarps within a few days.

On Difco potato-dextrose agar fascicles or tufts of aerial growth stand up from the surface. At first white, this growth becomes grayish to olivaceous and finally the culture turns

blackish because of the many perithecia formed in crust-like aggregations. On Difco dextrose agar the mycelium is white, the grayish-black appearance in older cultures being due to the presence of perithecia.

When ascocarps are formed on any medium discharge of ascospores may not begin until at the end of a month or even two months. They ooze from the ostiole and spread out about it in granular clumps. As the culture dries out the spores are discharged in cirri (PLATE 28, FIG. 4). Grown on different kinds of agar media, as well as on carrot, potato plugs and bread, no conidia have as yet been seen. Sopp (20) says of conidia of *Acaulium albo-nigrescens*: "In Kultur kann es selbst zu gewissen Jahreszeiten und auf einzelnen Nahrstoffen sogar immer schwer fallen, die Konidien zur Entwicklung zu bringen." Lechmere (14) says that the common form of *Peristomium desmosporum* did not produce conidia, but that a variety which he calls *P. desmosporum* var. *Verticillium* produced brown conidia which from his figures might be referred to *Scopulariopsis* notwithstanding their small size, 4  $\mu$ . He had a physiologic form of this variety which did not form conidia at ordinary temperatures, although it developed perithecia characteristic of *Peristomium*. At a higher temperature, 30° C., conidia just like those of the variety "*Verticillium*" were formed in abundance. Whether or not his type form without conidia is a *Microascus* and his variety *Verticillium* is a different species we have again another interesting parallel or correlation of forms.

Sopp's description of the origin and the growth of ascocarps of *Acaulium*, so far as he goes, corresponds very well with what Lechmere found for *Peristomium*, and their figures show a very clear relationship between their species. Whether Lechmere has given us the correct picture when he figures (14, 318, fig. 9) ascogenous hyphae growing out from the inner wall toward the center of the cavity is very doubtful. Unless one studies very young specimens he might confuse the upward inward growing rows of cells with ascogenous hyphae (see our PLATE 27, FIG. 3). There is no question that in both of our species the ascogenous hyphae beginning at the ascogonium well up under the ostiole grow out in all directions, downward and outward for the most

part, and directly back against the upward inward growing rows of sterile cells.

To assume that Sopp's technique was faulty and that the conidial stage he describes was that of a contaminant is not to be considered in light of his experience in culturing *Penicillia*. More likely, our fungus otherwise comparable to his species *Acaulium albo-nigrescens* is either a genotypically non-conidial race, or one which develops conidia only under very special conditions such as Lechmere found for his one physiologic form of *Peristomium*. The fact that our species does not develop ascocarps on Difco corn-meal agar but does so readily on ordinary corn-meal agar is proof of a very delicate balance as to nutritive conditions which might well dominate conidium production as well as peritheциum formation.

In spore discharge the end of the cirrus may bend down and become cemented to the spore mass about the ostiole, or to some other structure. By further discharge the cirrus, often as much as 4 mm. long, becomes looped and entangled with other cirri, giving the culture the characteristic appearance noted by Lechmere for *Peristomium*. Cirri are not usually formed until a culture has dried out considerably. Sopp might therefore have easily overlooked such a type of spore discharge in his cultures of *Acaulium*. His figure 59 in plate 7 which he says shows "entleerte Sporenmassen" is clearly of a fragment of a cirrus. He has given so many details as to cultural characters, and his measurements of perithecia, asci and spores correspond so well with ours, that we believe our fungus belongs to the same genus, but it is a distinct species and not a mere race or variety of his *Acaulium albo-nigrescens*. The method of the origin and development of the ascocarp in our species is the same as for *Microascus trigonosporus* and it is unnecessary to describe these details again.

Fraser and Chambers (9) state that the ascus of *Aspergillus herbariorum* sometimes arises from the binucleate cell of an ascogenous hypha whose uninucleate end cell turns back to complete the typical crosier. Dale (6) also found the same to be true for *A. repens*. Is it altogether logical to find asci of *Penicillium* borne in chains, as described by older authors, and

then find typical crosiers in *Aspergillus*? Can it be that the reasons usually held for throwing these two genera together in the same family are unsound morphologically? Relationships based on asexual stages, as noted previously, are not always confirmed by a study of the ascosporic fruiting structures. Material of neither species of *Microascus* studied by the writers is particularly suitable for a cytological study of the details of ascus formation. Very little evidence was found in the case of *M. trigonosporus* of the development of an ascus from the sub-terminal cell of a crosier. The tip ends of ascogenous hyphae of *M. intermedius*, however, were frequently seen bent somewhat in the form of a hook.

Rarely two or three ascogonia become involved in the organization of the same perithecium. In such cases two (or three) fertile regions, each independent of the other, result. Figure 3, plate 28, shows a section of a fruit body of this kind which happened to be cut so that only one group of ascogenous hyphae with the corresponding ostiolar portion appears in the section. At the lower left are seen the cut ends of the rows of sterile cells which are pointing toward a second set of ascogenous elements, visible several sections farther along in the series. Ordinarily there will be developed a distinct ostiole for each fertile region, but in the case shown here the second ostiolar structure for some reason was started on the wrong side of the perithecium and failed to mature. Undoubtedly here, as in other cases observed, the sterile tissue separating the fertile regions will disorganize so that all of the ascospores will be discharged through the same ostiole. Very well marked cases have been seen where three distinct and functional ostioles were formed by the same perithecium. A fruit body with two fertile regions and two ostioles which show in the same section are pictured in plate 28, figure 6.

The outer carbonized wall increases in circumference by elongation of its original elements and by intercalary growth, the lower portion growing more rapidly. With no corresponding growth of the central elements a schizogenous cavity would result. The thin-walled cells which surround the ascogonium in the early stages of perithecium development increase in size

up to a certain point, then as space is provided they elongate inwardly, becoming septate, tapering more and more as they approach the ascogonium, which, as noted previously, is now located above the center of the ascocarp or beneath the ostiole (PLATE 28, FIGS. 1, 2). Ascogenous hyphae and asci can develop only at the expense of this sterile tissue. The whole central cavity, then, which at the maturity of the perithecium is filled with asci and freed spores, is formed through disorganization of sterile tissue which at first grew in to fill the space provided by inequalities of growth. In principle the central cavity of a mature perithecium originates lysogenetically but only indirectly, because of the disorganization of cells developed more or less to fill a space being provided by forces working schizogenetically.

#### SUMMARY

The writers have studied in culture an ascomycete having a *Scopulariopsis* conidial stage and an ascocarp stage which corresponds to *Microascus*, except perhaps that it has triangular ascospores, whereas *Microascus sordidus* has kidney-shaped spores. This new species is described as *Microascus trigonosporus*, and its conidial stage is referred to as *Scopulariopsis trigonospora*. The ascocarp arises from a coiled ascogonium which becomes enveloped in a hyphal weft of several layers of cells. Later this envelope becomes differentiated into an outer wall of dark-colored carbonized cells and an inner portion consisting of thin-walled colorless cells. The cells immediately surrounding the ascogonium begin to elongate inwardly, crowding in to fill up the space made available because of intercalary growth of the outer wall. A papillate ostiolar portion is then organized and its cavity formed schizogenetically. Because the outer wall increases in its circumference more rapidly below and at the sides than at the top, and because the inward growing hyphae develop more rapidly from below than from above, the ascogonium becomes placed well above the center and just beneath the ostiole. Most of the growth of the thin-walled cells is upward and inward. The ascogenous hyphae sometimes grow out from the ascogonium in all directions, but usually most of the growth, because of the position of the ascogonium, is outward

and downward, and, therefore, back against the upward inward growing rows of sterile cells. These sterile cells are gradually absorbed and their place in the cavity is taken by ascospores set free as the asci deliquesce. The ascospores are discharged in long slender cirri containing a cementing substance which hardens on drying and which then is dissolved in water only very slowly.

*Microascus intermedius* develops only ascocarps which in their organization, in general, correspond very well with *Microascus* of Zukal and, except for the absence of a conidial stage, it resembles Sopp's *Acaulium albo-nigrescens*. These two species, because of the *Scopulariopsis* conidial stage of one, and because of their black carbonaceous ascocarps with ostioles, and the growth of the ascogenous hyphae outward from the ascogonium in all directions, seem to make the series including *Penicillium*, *Aspergillus*, *Thielavia*, *Scopulariopsis*, *Microascus*, *Acaulium*, *Peristomium*, *Nephrospora* and *Chaetomium*, more complete.

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#### EXPLANATION OF PLATES

##### PLATE 25

*Microascus trigonosporus*. Fig. 1. Conidiophores and conidia; 2. Conidiophores with partial whorls of sterigmatin, conidia, and germinating conidia; 3. Stages in the formation of the ascocarp; 4. Ascogenous hyphae and young ascii; 5. Ascogenous hyphae and young ascii in some of which spores are partially formed; 6. Ascii and spores; a, eight-spored ascii; b, ascospores; c, germinating ascospore. Fig. 1,  $\times 500$ ; Figs. 2-6,  $\times 1400$ .

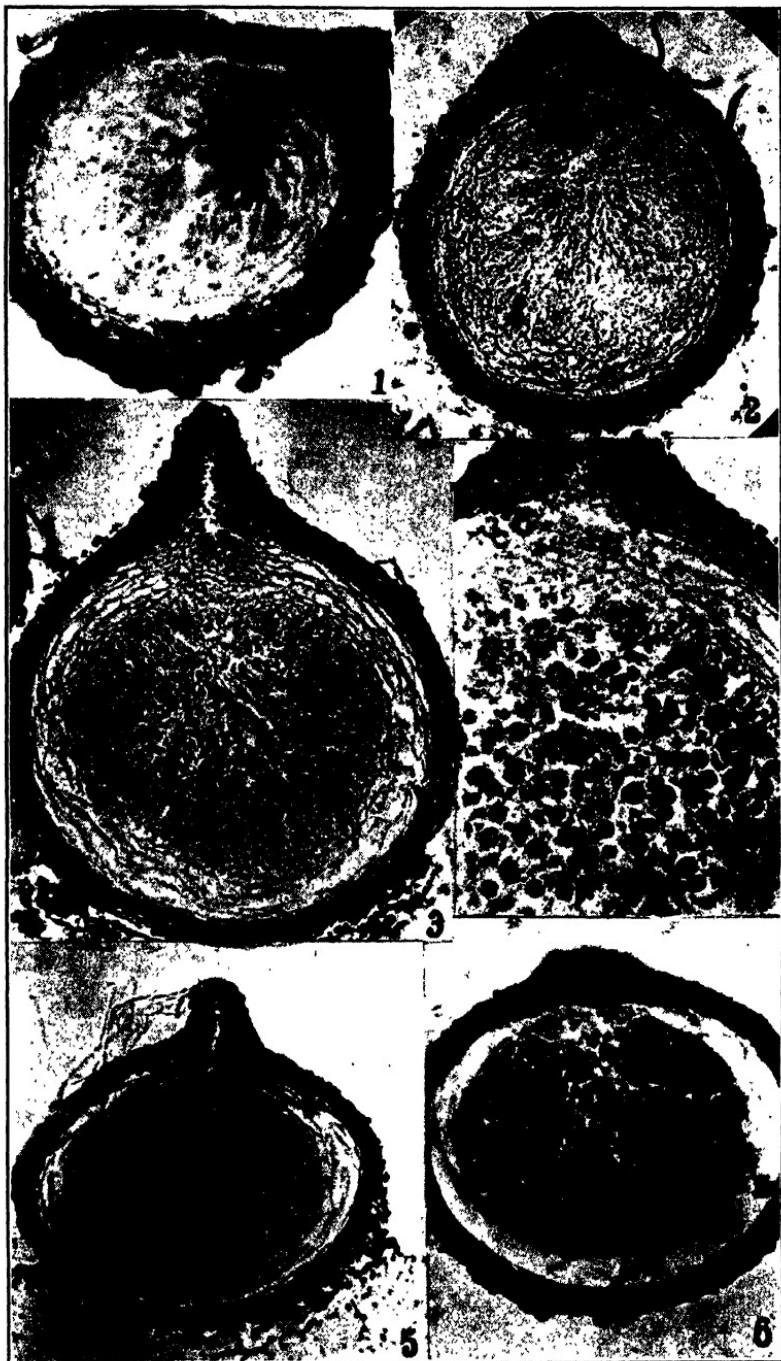
##### PLATE 26

*Microascus trigonosporus*. Fig. 1. Young ascocarp showing two cells of the ascogonium; one cell of which, at this level, contains four nuclei; 2. Later stage in which multinucleate cells of the ascogonium are shown above a region where the cells are elongating inward. Median section; 3. Median longitudinal section of an ascocarp in which the upward growth of sterile tissue is more fully developed; 4. Median longitudinal section of an ascocarp in a



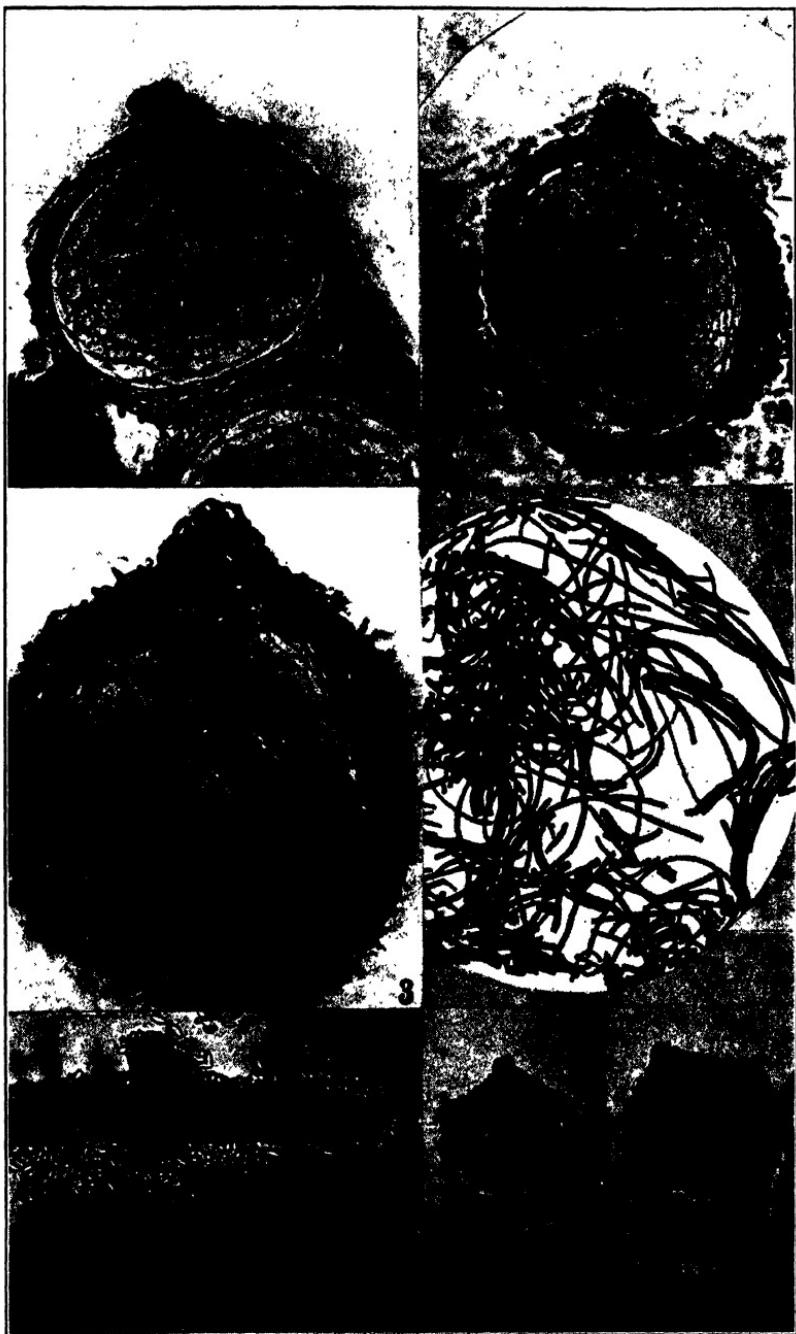
MICROASCUS TRIGONOSPORUS





MICROASCUS TRIGONOSPORUS





*MICROASCUS INTERMEDIUS*



stage similar to that of the one shown in Fig. 3; 5. Stage of development slightly later than that shown in figure 4. The ascogenous hyphae are just beginning to develop; 6. Ascogenous hyphae have begun to grow downward from the ascogonium, pushing against and in between the rows of sterile cells. The ostiolar development has begun. Fig. 1,  $\times 675$ ; Figs. 2-6,  $\times 575$ .

#### PLATE 27

*Microascus trigonosporus*. Fig. 1. Median longitudinal section of an ascocarp showing downward growth of ascogenous hyphae. The developing ostiole is in the upper right corner. A part of the ascocarp has been cut away.  $\times 675$ ; 2. Median section of an ascocarp in which the ascogenous hyphae have grown far down into the sterile tissue. The ostiolar cavity is well developed.  $\times 400$ ; 3. The ascogenous hyphae have reached the perithecial wall at the base, a few asci already formed in the upper half of the ascocarp. The short periphyses are apparent in the neck of the ostiole.  $\times 400$ ; 4. Portion near the edge of an ascocarp showing asci.  $\times 1200$ ; 5. Ascogenous hyphae radiating from the ascogonium.  $\times 350$ ; 6. Section of a mature ascocarp filled with asci. The short neck of the ostiolar structure was not in focus.  $\times 400$ .

#### PLATE 28

*Microascus intermedius*. Fig. 1. Section of a young ascocarp showing ostiolar structure and the ascogonium well above the center and just beneath the ostiole. Ascogenous hyphae growing out from the ascogonium and down against the sterile upward growing rows of cells.  $\times 400$ ; 2. Section of a somewhat older ascocarp, ascogenous hyphae growing out in all directions.  $\times 400$ ; 3. At the center above are shown deeply staining ascogenous hyphae growing out from the ascogonium just beneath the ostiole. Lower left, cut ends of rows of sterile cells such as are seen in longitudinal section in Fig. 1. Two ascogonia were involved in the origin of this ascocarp; the second set of fertile hyphae appeared several sections farther along in the series.  $\times 450$ ; 4. Ascocarp with two separate sets of ascogenous hyphae and two functional ostioles.  $\times 200$ ; 5. Two ostioles and three sets of ascogenous hyphae.  $\times 200$ ; 6. Fragments of cirrhi after soaking in water several hours.  $\times 90$ ; 7. Fragment of cirrhus and ascospores.  $\times 600$ .

# THE RUSTS OF SOUTH AMERICA BASED ON THE HOLWAY COLLECTIONS—IV<sup>1</sup>

H. S. JACKSON

(WITH 6 TEXT FIGURES)

## SPECIES ON LEGUMINOSAE—MIMOSOIDEAE

100. DIORCHIDIUM PIPTADENIAE Dietel, Hedwigia **38**: 252.  
1899.

*Puccinia papillifera* Sydow, Monog. Ured. **1**: 836. 1904.  
*Diorchidium brasiliense* Arth. Bull. Torrey Club **51**: 54.  
1924.

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*Piptadenia latifolia* Benth. Rio de Janeiro, Brazil,  
Aug. 9, 1921, 1009.

*Piptadenia laxa* Benth. Petropolis, Rio de Janeiro,  
Brazil, Oct. 23, 1921, 1244; Jacarépaguá, Rio de Ja-  
neiro, Brazil, Nov. 16, 1921, 1316; Barbacena, Minas  
Geraes, Brazil, Dec. 12, 1921, 1385; Nova Friburgo,  
Rio de Janeiro, Brazil, Jan. 1, 1922, 1437; Garulhos,  
Brazil, June 1, 1922, 1929.

We have assigned all the above collections to this species  
with some hesitation. Dietel described uredinia for the species.  
We find uredinia abundant on two of the collections (1316 &  
1929) on *Piptadenia laxa*. Arthur described *D. brasiliense* from  
a portion of collection 1009 as a microcyclic species. We find  
urediniospores with the telia on duplicate specimens of this  
collection. The host of this collection was originally considered  
to be *Cassia* sp. It has since been identified as *Piptadenia*

<sup>1</sup> Joint contribution from the Department of Botany, Purdue University Agricultural Experiment Station, and the Department of Botany, University of Toronto. Prepared with the aid of a grant from the American Association for the Advancement of Science. This is the fourth of a series of papers bearing the same title. (See *Mycologia* **18**: 139-162. 1926; **19**: 51-65. 1927; **23**: 96-116. 1931.)

Drawings and photomicrographs in this and the preceding number in this series were prepared by Miss Lillian M. Hunter.

*latifolia* which is the host for Dietel's type. There exists on *Piptadenia*, *Puccinia Piptadeniae* P. Henn., which may be a *Diorchidium*. Hennings described uredinia for this species.

Arthur, as above noted, described his species as a microcyclic form with subcuticular pycnia. It would appear that there are two similar species of different life cycle, one a brachy-form and the other a micro-form, or it is possible that there is one mutable species. In assigning the collections to one species we have taken the latter view.

#### 101. *Dichaerina superba* Jackson & Holway, sp. nov.

O. Pycnia amphigenous or caulicolous, few, inconspicuous among the telia or grouped in the centre of a group of telia, subcuticular, flattened hemispheric, 20–30  $\mu$  high by 90·150  $\mu$  broad, opening irregularly.

III. Telia amphigenous, caulicolous or petiolicolous, small, numerous, pulverulent, chestnut-brown, on hypertrophied areas 2–3 cm. long, when on stems or petioles; when on leaves occurring closely gregarious or confluent in circular or irregular groups 2–8 mm. across, often involving the veins and then spots more elongate; paraphyses few, occurring either at periphery or scattered throughout sorus, irregularly cylindrical, thin-walled, colorless; teliospores two-celled with vertical septum, 20–25  $\mu$  high by 22–28  $\mu$  broad, usually noticeably constricted between cells above, often less so below; wall uniformly thin 1–1½  $\mu$ , cinnamon-brown, adorned by conspicuous hyaline or tinted tubercles of irregular or elongate outline which occur abundantly scattered at apex of spore becoming less abundant below, often arranged in oblique lines with smooth areas between; pedicel short, deciduous, colorless, with two small cells at distal end on which are borne the two teliospore cells placed side by side.

*Inga* sp. Petropolis, Rio de Janeiro, Brazil, Oct. 20, 1921,  
1234 type.

This is a conspicuous species occurring on all parts of the host, apparently becoming more or less locally systemic in the younger tissues and then causing considerable hypertrophy.

There would seem to be no doubt that this form is strictly microcyclic as pycnia occur consistently with the telia.

The type species of the genus, *Dichaerina binata* (Berk.) Arth., is described with uredinia. The pycnia are unknown but presumably subcuticular as in the species described above.

The two basal cells are easily overlooked in the mature spores, as they are usually collapsed. They are easily demonstrated in the young spores, however, and can often be detected in the mature ones. (FIG. 1.)

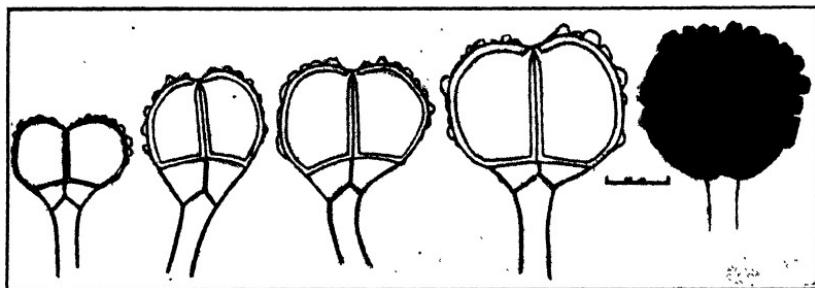


FIG. 1. Teliospores of *Dichaerina superba*. Those at the left are only partially developed. The two small basal cells are easily overlooked in fully mature spores.

**102. RAVENELIA ECHINATA** Lagerh. & Dietel; Dietel, *Hedwigia* **33**: 65. 1894. \_\_\_\_\_

*Calliandra* sp. Guayaquil, Ecuador, July 31, 1920, 804.

The type of this species was collected in Ecuador, in 1891, by Lagerheim and does not appear to have been reported elsewhere.

**103. RAVENELIA ECTYPA** Arth. & Holway, *Mycologia* **10**: 120. 1918. \_\_\_\_\_

*Calliandra laxa* Benth. Sorata, Bolivia, Apr. 14, 1920, 520.

This species has been previously reported only from Guatemala and Costa Rica. The single collection seems to agree well with the type. The species differs from *R. echinata* Lagerh. & Dietel primarily in the size and the number of cells in the teliospore-heads. The urediniospores are similar.

**104. RAVENELIA HENNINGSIANA** Dietel, *Beih. Bot. Centr.* **20**: 388. 1906. \_\_\_\_\_

*Piptadenia* sp. Rio de Janeiro, Brazil, Aug. 23, 1921, 1066; Sept. 11, 1921, 1101.

105. **RAVENELIA HIERONYMI** Speg. Anal. Soc. Ci. Argent. **12**:  
66. 1881.

*Aecidium Hieronymi* Speg. Anal. Soc. Ci. Argent. **12**: 78.  
1881.

*Ravenelia Mimosae* P. Henn. Hedwigia **34**: 95. 1895.

*Ravenelia Acaciae-farnesiana* P. Henn. Hedwigia **34**:  
321. 1895.

*Cystingophora Hieronymi* Arth. N. Am. Fl. **7**: 131. 1907.

*Acacia Farnesiana* (L.) Willd. Banos de Cauquenes,  
Rancagua, Chile, Jan. 13, 1920, 295; Papudo, Chile,  
Feb. 2, 1920, 313.

A common species of wide distribution in South and Central America. The above listed collections consist of aecia only.

106. **Ravenelia idonea** Jackson & Holway, sp. nov.

O. Pycnia not seen.  
II. Uredinia amphigenous, chiefly epiphyllous, subcuticular, scattered, round, small, cinnamon-brown, pulverulent, soon naked, ruptured epidermis conspicuous; paraphyses few, cylindrical or clavate, colorless, thin-walled, apex slightly thickened; urediniospores globoid, broadly or narrowly ellipsoid according to view, compressed laterally, 18-20 by 20-22  $\mu$  or 12-14 by 20-22  $\mu$ ; wall cinnamon-brown, equal, thin, 1.5-2  $\mu$ , closely and prominently echinulate, pores 4, approximately equatorial.

III. Telia like the uredinia; teliospore heads compressed globoid, chestnut-brown, 45-75  $\mu$  in diameter, 25-30  $\mu$  high, composed of 10-22 cells with 5-7 central cells, smooth; cysts globoid, numerous, pendent from outer cells, not readily bursting in water; pedicel colorless, compound.

*Acacia riparia* H.B.K. Santa Anna, São Paulo, Brazil,  
May 25, 1922, 1879 (type).

*Acacia* sp. Nictheroy, Rio de Janeiro, Brazil, Aug. 18,  
1921, 1055; Rio de Janeiro, Brazil, Sept. 14, 1921,  
1112.

*Mimosa sepiaria* Benth. Rio de Janeiro, Brazil, Aug. 12,  
1921, 1028.

107. **RAVENELIA INGAE** (P. Henn.) Arth. N. Am. Fl. **7**: 132.  
1907.

*Uredo Ingae* P. Henn. Hedwigia Beibl. **38**: 69. 1899.

*Uredo excipulata* Sydow, Ann. Myc. 2: 350. 1904.

*Uromyces ingicola* P. Henn. Hedwigia 43: 157. 1904.

*Uromyces porcensis* Mayor, Mém. Soc. Neuch. Sci. Nat. 5: 459. 1913.

*Hauploravenelia Ingae* Sydow, Ann. Myc. 19: 165. 1921.

*Inga edulis* Mart. Rio de Janeiro, Brazil, Aug. 13, 1921, 1031.

*Inga insignis* H.B.K. Valle Chiche, Quito, Ecuador, Sept. 3, 1920, 962.

*Inga* sp. Petropolis, Rio de Janeiro, Brazil, Oct. 20, 1921, 1235; Pinheiros, São Paulo, Brazil, March 27, 1922, 1684; Alto da Serra, Brazil, June 14, 1922, 1968.

According to the interpretation of Arthur (N. Am. Fl. 7: 707, 1925) this species possesses two quite distinct types of uredinia. Collections 962, 1684 and 1968 are primary uredinia with markings in more or less spiral striae while collections 1031 and 1235 are of the secondary type, which is often systemic in the stems and in which the uredospores are smaller and the wall echinulate. Culture work will be necessary to determine whether or not these two types belong to the same species. Telia are unknown for the species.

108. **Ravenelia irregularis** Jackson & Holway, sp. nov.

O. Pycnia not seen.

II. Uredinia epiphyllous, subepidermal, numerous, scattered, cinnamon-brown, pulverulent, ruptured epidermis noticeable; paraphyses not seen; urediniospores ellipsoid, obovate or pyriform, often irregular 10–12 by 18–26  $\mu$ , wall thin, 1–1.5  $\mu$  cinnamon-brown, slightly or not at all thickened at the apex, finely and moderately echinulate; pores 5 approximately equatorial.

III. Telia epiphyllous, subepidermal, scattered, chestnut-brown, soon naked, ruptured epidermis noticeable; teliospore heads chestnut-brown, made up of 10–22 cells, flattened globoid, 35–75  $\mu$  wide, 30  $\mu$  high, each cell with few, 4–8 hyaline or slightly tinted conical projections 2–3  $\mu$  high; cysts numerous, pendent.

*Acacia* sp. Rio de Janeiro, Brazil, Aug. 23, 1921, 1065; Dec. 20, 1921, 1416 (type); *Sylvestre*, Rio de Janeiro, Brazil, Sept. 14, 1921, 1109.

109. RAVENELIA LAGERHEIMIANA Dietel, Hedwigia 33: 65.  
1894.

*Calliandra falcata* Benth. Huigra, Prov. Chimborazo,  
Ecuador, Aug. 3, 1920, 822.

The type collection of this species, which seems to be the only previous one, was made by Lagerheim in the same Province of Ecuador. The host is probably the same.

110. RAVENELIA LEUCAENAE-MICROPHYLLAE Dietel, Beih. Bot. Centr. 20: 375. 1906.

*Acacia* sp. Gavea, Rio de Janeiro, Brazil, Sept. 8, 1921,  
1100.

The type locality for this species is in Mexico. The host was originally determined as *Leucaena microphylla* Benth., now identified as *Acacia angustissima* (Mill.) Kuntze. We have identified the collection as listed above because of the large urediniospores with 4 equatorial pores, the capitate paraphyses and large smooth teliospore heads.

111. RAVENELIA MIMOSAE-ALBIDAE Dietel, Beih. Bot. Centr. 20: 378. 1906.

*Mimosa albida* H. & B. Chosica, Peru, July 22, 1920, 780.

This species has apparently not been reported previously from South America. The type was collected in southern Mexico and only a few collections are known from that locality.

112. **Ravenelia rata** Jackson & Holway, sp. nov.

O. Pycnia amphigenous, chiefly epiphyllous, subcuticular, in small crowded groups, chestnut-brown, flattened hemispheric, 75–135  $\mu$  broad, 30–45  $\mu$  high.

III. Telia amphigenous, subepidermal in small groups among the pycnia, 1–2 mm. across, cinnamon-brown, soon naked, ruptured epidermis not conspicuous; teliospore heads cinnamon-to chestnut-brown, made up of 7–9 cells, irregularly flattened globoid, 30–45  $\mu$  wide, each cell with 8–10 hyaline, irregular tubercles 2–3  $\mu$  high; cysts 2–4 subgloboid, small and inconspicuous, evidently related to central cells of the head only; pedicel short, colorless, usually deciduous.

*Acacia pedicellata* Benth. Rio de Janeiro, Brazil, Aug. 13, 1921, 1032 (type); Sept. 14, 1921, 1110.

This apparently distinct microcyclic form is without paraphyses. It would be placed in the genus *Dendroecia* of the Arthurian classification based on differences in life cycle. A feature noticed in connection with a study of sections seems worth recording. In the infected spots the upper epidermis is apparently raised above the palisade cells by the development of a weft of mycelium 10–15  $\mu$  thick. This weft extends slightly beyond the group of pycnia and telial sori. The leaf is therefore thickened not through hyperplasia but rather from the interpolation of a layer of tangled mycelium beneath the epidermis.

SPECIES ON LEGUMINOSAE—CAESALPINIOIDEAE

**Mimema** Jackson, gen. nov.

Pycnia and aecia unknown, the former probably subcuticular, the latter uredinoid. Uredinia and telia stilosporic. Teliospores three or more celled by the development of transverse septa. Somewhat parallel to *Hamaspora* but developing on Leguminosae.

113. **Mimema Holwayi** Jackson, sp. nov.

II. Uredinia hypophyllous, subepidermal, deep seated, scattered, small, .3–.5 mm. across, soon naked, whitish from the abundant paraphyses, becoming pulverulent, and cinnamon-brown from the urediniospores, ruptured epidermis not noticeable; paraphyses abundant, forming a dense fringe about the sorus, at first colorless becoming brownish due to deposit of color in contents, irregularly cylindric, 5–8 by 30–55  $\mu$ , pointed at tip, incurved, free or united to a compact tissue at base, wall colorless, thin, 1–1½  $\mu$  on inner side, irregularly thickened 2.5–5  $\mu$  on outer side, often solid at pointed tips; urediniospores ellipsoid or obovate 15–18 by 19–22  $\mu$ , wall 1.5–2  $\mu$  thick, finely and closely echinulate-verrucose, pores obscure, 4–6 in a super-equatorial zone.

III. Teliospores apparently following the urediniospores in the same sorus or one of similar type; cylindrical or fusiform, 8–10 by 75–105  $\mu$  long, composed of 3–5 cells, usually 4, the upper and lower cells usually longer than the middle ones, slightly constricted at the septa, rounded or obtuse above, tapering to

pedicel below; wall colorless, uniformly thin, 1  $\mu$  or less; pedicel colorless, 30–75  $\mu$  long, 8  $\mu$  broad above, tapering below.

*Cassia versicolor* Mey.? Villa Aspiazú, Sur Yungas, Bolivia, May 31, 1920, 690 (type); Hacienda "Anacuri," Nor Yungas, Bolivia, June 4, 1920, 715.

This very interesting species is made the type of a new genus on grounds which we fully realize may not be acceptable to all uredinologists. In his recently revised generic classification of the Uredinales Dietel (in Engler, Die Natürlichen Pflanzenfamilien, Ed. 2, 6: 24–98. 1928) has made use of the principle that the rusts have evolved with their hosts and that forms of similar morphology may appear independently in quite unrelated series. As an example one may take Dietel's treatment of the old genus *Triphragmium*. The species formerly included in this genus are now to be found in four genera in two tribes: *Triphragmium* in the Phragmidieae; *Triactella*, *Triphragmiopsis* and *Nyssopsora* in the Ravenelieae.

According to this treatment the Phragmidieae include a group of more or less closely related genera which occur on and have evolved with the members of the host family Rosaceae, while the Ravenelieae, on the other hand, have developed most abundantly, though not exclusively, on the Leguminosae. While in general the Phragmidieae have tended to the development of a series of genera characterized by transverse septa in the teliospores, the development in the Ravenelieae has centered primarily in the variations made possible by the development of longitudinal septa, culminating in *Ravenelia*. It would appear, however, that forms with transverse septa have developed in this series also.

This classification seems to the writer to approach more closely to a phylogenetic system than any that has previously been proposed and while such a system must be recognized as, in a sense, experimental, it seems worth while to give it a thorough trial. Many more forms remain to be discovered and it may be that when these are available for comparative study such a classification will prove unworkable. Dietel's classification, however, is likely to be adopted as the standard for some time to come, and it seems desirable in describing new forms to fit them into this

system. For these reasons the generic name *Mimema* has been provided for this species and others which may be later found to belong here.

We fully recognize that this genus parallels *Hamaspora* rather closely. *Hamaspora*, however, is known only on the genus

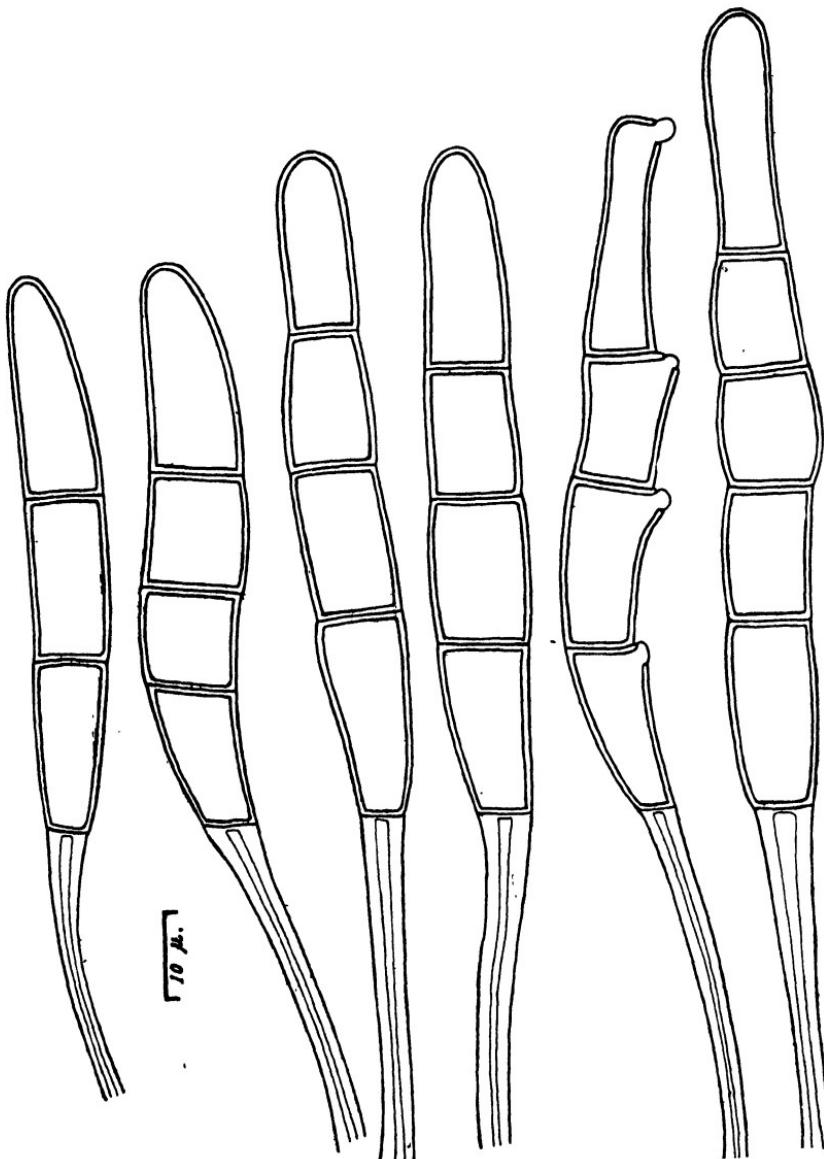


FIG. 2. Teliospores of *Mimema Holwayi*. Compare with figure 6.

*Rubus* of the Rosaceae and is included in the Phragmidieae by Dietel. A comparison of our species with several collections of *Hamaspora* leads to the conviction that the two rusts are not immediately related but that they have developed along parallel lines in unrelated series. We believe that *Mimema* should be included in the Ravenelieae for the present. For the benefit of those who believe that such an attempt at generic classification is premature and unwarranted, the name **Hamaspora Holwayi** comb. nov. might be used.

The teliospores (FIG. 2) apparently germinate at the apex and just below each septum, though no very evident pores are visible until germination has begun. The paraphyses form a dense fringe about the sorus. The latter in dried specimens appear white at first but become brown later apparently due primarily to the contents, though occasionally the wall may be tinted golden brown. The urediniospores are produced in great abundance and accumulate on the surface of the leaf.

#### 114. *Triactella Holwayi* Jackson, sp. nov.

II. Uredinia hypophyllous, scattered, small, round, 0.2-0.4 mm. across, cinnamon brown, early naked, pulverulent, ruptured epidermis not conspicuous; paraphyses abundant, encircling the sorus, mostly arcuate, short, 25-38  $\mu$  long by 6.5-9  $\mu$  broad; the wall colorless or golden brown and somewhat irregularly thickened on both sides, often obliterating the lumen; urediniospores obovoid or broadly ellipsoid, 12-14 by 17-20  $\mu$ ; wall colorless, 1-1.5  $\mu$  thick, very finely and closely echinulate, the pores 2-3 equatorial.

III. Telia like the uredinia, chestnut brown; paraphyses apparently as in the uredinia; teliospores three celled as in *Triphragmium*, nearly circular in outline when in face view, 24-26  $\mu$  by 25-26  $\mu$ , compressed and appearing considerably narrower when in side view; wall cinnamon brown, 2-2.5  $\mu$  thick, slightly thickened at angles, prominently echinulate-tuberculate with conical markings up to 6  $\mu$  high; pedicel colorless, short,  $\frac{1}{2}$  the length of the spore or less, attached at the center of the side wall of lower cell; pores obscure, apparently one in each cell.

*Cassia* sp. Tijuca, Rio de Janeiro, Brazil, Dec. 23, 1921,  
1419.

The genus *Triactella* Syd. is retained in the Ravenelieae of the recently revised classification of Dietel (l.c.) for those species of

*Triphragmium* having one germ pore in each cell, and which occur on Leguminosae. The type species for *Triactella* and the only one previously assigned to this genus is *T. pulchra* (Rac.) Sydow, which occurs in Java on *Derris elliptica*.

The teliospores in the species described above are characteristic (Fig. 3). Two celled teliospores occur fairly commonly, and rarely

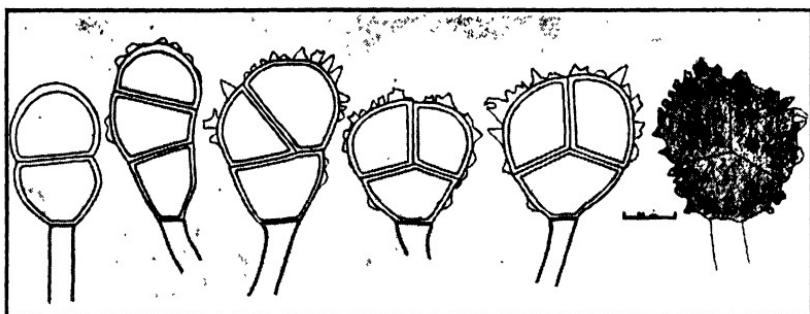


FIG. 3. Teliospores of *Triactella Holwayi* with variations. The three celled *Triphragmium*-like spores are typical.

the septum which separates the two upper cells is formed transversely. It is possible that *Uredo cassiicola* P. Henn. is the same, though paraphyses are not described. No specimens have, however, been available for comparison.

#### 115. *Ravenelia faceta* Jackson & Holway, sp. nov.

O. Pycnia not seen.

II. Urediniospores in the telia ellipsoid or obovate, 26–30 by 18–21  $\mu$ , wall uniformly  $1\frac{1}{2}$ –2  $\mu$  thick, chestnut-brown, very finely and closely verrucose, pores 4 equatorial.

III. Telia hypophylloous, subepidermal, scattered, minute, chestnut-brown, soon naked, ruptured epidermis evident; paraphyses numerous, scattered but chiefly peripheral, colorless; scattered paraphyses cylindrical or clavate, slightly thickened at apex; peripheral paraphyses incurved, somewhat irregularly cylindrical, thickened on the outer side; teliospore heads chestnut-brown 45–60  $\mu$  broad, composed of 8–12 cells, each bearing on outer wall numerous tinted appendages 3–6  $\mu$  high, lobed or tuberculate at apex; cysts colorless, few, 3–5, mostly 4, pendulous, small, globose, 15  $\mu$  in diameter, not readily bursting in water; pedicel colorless, compound, usually deciduous.

*Cassia* sp. Jacarépaguá, Rio de Janeiro, Brazil, Sept. 4, 1921, 1091.

116. **RAVENELIA MACROCARPA** Sydow, Ann. Myc. 1: 329. 1903.

*Cassia bicapsularis* L. Sylvestre, Rio de Janeiro, Brazil,  
Aug. 15, 1921, 1038.

117. **RAVENELIA MICROSPORA** Dietel, Ann. Myc. 6: 98. 1908.

*Cassia excelsa* Schrad. Petropolis, Rio de Janeiro, Brazil,  
Oct. 21, 1921, 1239; Rio de Janeiro, Brazil, Dec. 20,  
1921, 1415; Pinheiros, São Paulo, Brazil, March 27,  
1922, 1682; São João, São Paulo, Brazil, July 2,  
1922, 1990.

A very distinct species separable from other species on *Cassia* by the minute urediniospores. It has been previously reported only from the type locality at Nossa, Lenhora, São Paulo, Brazil. The last collection listed above (1990) includes uredinia and telia, the others uredinia only.

118. ? **UROMYCES DIETELIANUS** Pazschke, Hedwigia 30: 199,  
1891.

*Bauhinia* sp. Petropolis, Rio de Janeiro, Brazil, Oct. 16.  
1921, 1225.

119. ? **UROMYCES FOVEOLATUS** Juel, Bih. Svernsk. Vet.-Akad.  
Handl. 23: 16. 1897.

*Bauhinia* sp. Fonseca, Nictheroy, Rio de Janeiro, Brazil,  
Sept. 18, 1921, 1120; Petropolis, Rio de Janeiro,  
Brazil, Nov. 3, 1921, 1270; Mogy das Cruzes, Brazil,  
July 4, 1922, 1998.

120. ? **UROMYCES HEMMENDORFFII** Vesterg. Arkiv. för Bot. 4:  
32. 1905.

*Bauhinia* sp. Rio de Janeiro, Aug. 12, 1921, 1025; Man-  
daqui, São Paulo, Brazil, May 25, 1922, 1883.

This species is distinguished by the small teliospores and the moderate thickening of the wall.

121. ? **UROMYCES PERLEBIAE** Vesterg. Arkiv. för Bot. 4: 28.  
1905.

*Bauhinia forficata* Link. São João, São Paulo, Brazil,  
March 19, 1922, 1652.

*Bauhinia forficata latifolia* Benth. Prata, São Paulo,  
Brazil, Apr. 7, 1922, 1702.

This species was originally described from Brazil and seems not to have been reported elsewhere. Specimen 1702 bears pycnia with primary uredinia showing that this species is a brachy-form. These are epiphyllous, pyriform or ellipsoid, deep-seated, 90–105  $\mu$  high by 90–100  $\mu$  broad, without ostiolar filaments.

122. *Uromyces verus* Jackson & Holway, sp. nov.

O. Pycnia epiphyllous, few, deeply seated, punctiform, closely grouped among telia, ellipsoid or pyriform 125–150  $\mu$  high by 100–135  $\mu$  wide, ostiolar filaments not noticeable.

III. Telia amphigenous or caulicolous, abundant, scattered or more commonly gregarious, often confluent on discolored spots, cinnamon-brown, soon naked, pulverulent, ruptured epidermis conspicuous particularly when epiphyllous, when caulicolous, extending for considerable areas along younger stems; teliospores ellipsoid or obovoid, 15–18  $\mu$  by 21–25  $\mu$ , rounded above and below; wall golden or light cinnamon-brown 2 or 3  $\mu$  thick, slightly thickened 4–6  $\mu$  at apex often with subhyaline umbo, closely uniformly and finely verrucose; pedicel colorless, short deciduous.

*Bauhinia rufa* Stend. Bello Horizonte, Minas Geraes, Brazil, Nov. 21, 1921, 1319 (type).

This micro-form with verrucose spores seems to be amply distinct. No other micro-form with this type of spore marking seems to have been described on *Bauhinia*.

SPECIES ON LEGUMINOSAE-PAPILIONATAE (FABACEAE)

123. *AECIDIUM DESMODII* P. Henn. *Hedwigia* 35: 259. 1896.

*Desmodium uncinatum* Sw. Taipas, São Paulo, Brazil, Feb. 7, 1922, 1544; Campo Grande, Rio de Janeiro, Brazil, Sept. 19, 1921, 1127.

*Desmodium* sp. Sorata, Bolivia, Apr. 15, 1920, 526a; Therezopolis, Rio de Janeiro, Brazil, Sept. 28, 1921, 1163; Bello Horizonte, Minas Geraes, Brazil, Nov.

30, 1921, 1351; Nova Friburgo, Rio de Janeiro, Brazil, Jan. 3, 1922, 1450; Alto da Serra, São Paulo, Brazil, Jan. 28, 1922, 1505; Cantareira, São Paulo, Brazil, Feb. 18, 1922, 1567a.

This aecidium appears to be common especially in southern Brazil. While it has been commonly assumed to be the aecial stage of *Uromyces Hedysari-paniculati*, Holway repeatedly makes note that it is not followed by any other stage. He apparently believed it to be heteroecious. The aeciospores are smaller than usually described for the *Uromyces*.

Since there seems to be some doubt about the connection we have listed the aecidial collections separately from those of the *Uromyces*.

124. CHRYSOCELIS LUPINI Lagerh. & Dietel in Mayor, Mem. Soc. Neuch. Sci. Nat. 5: 542. 1913.

*Lupinus paniculatus* Desv. Riobamba, Ecuador, Aug. 10, 1920, 861; Quito, Ecuador, Aug. 18, 1920, 916; Aug. 19, 1920, 921.

*Lupinus* sp. along railway, foot of Cotapaxi, Ecuador, Aug. 12, 1920, 872; Quito, Ecuador, Aug. 19, 1920, 919.

This very interesting and characteristic species was originally described from Colombia. It was first collected in Ecuador by Lagerheim and is known otherwise only from Costa Rica. Arthur (N. Am. Fl. 7: 663, 1924) places this genus in a special tribe Chrysoceliatae of the Uredinaceae (Melampsoraceae) while Dietel (Engler, Nat. Pflanzenfamilien, II. Auflage 6: 48. 1928) places it in the tribe Oliveae of the Pucciniaceae. I would favour its inclusion in the latter family.

125. RAVENELIA PLATENSIS Speg. Anal. Mus. Nac. Buenos Aires 6: 228. 1899.

*Erythrina Crista-galli* L. Petropolis, Rio de Janeiro, Brazil, Nov. 9, 1921, 1288.

*Erythrina* sp. Therezopolis, Rio de Janeiro, Brazil, Sept. 28, 1921, 1157.

This species appears to be known previously only from several collections made in Argentina and reported by Spegazzini. The collections listed above show primary uredinia only. These do not appear to have been previously distinguished. The sori occur on stems, petioles and leaves, often causing considerable hypertrophy and covering considerable areas. The pycnia are subcuticular, when on leaf blades amphigenous, flattened hemispheric, 40–60  $\mu$  high by 75–115  $\mu$  wide, often partially laterally confluent. The primary uredinia are amphigenous, irregular and confluent, often circinating about the pycnia. Paraphyses appear to be wanting. The urediniospores are broadly ellipsoid or obovoid, 25–28 by 30–35  $\mu$ , wall light chestnut brown, 3.5–5  $\mu$  thick, sparsely and very prominently echinulate, the pores three, approximately equatorial.

126. HAPLOPYXIS CROTALARIAE (Arth.) Sydow, Ann. Myc. 17: 105. 1919.

*Uropyxis Crotalariae* Arth. Am. Jour. Bot. 5: 429. 1918.

*Crotalaria vitellina* Ker. Sylvestre, Rio de Janeiro, Brazil, Sept. 16, 1921, 1114.

This collection which consists of uredinia only seems to agree best with the above species. The rust has hitherto been reported only from Guatemala.

127. PHAKOPSORA CROTALARIAE (Dietel) Arth. Bull. Torrey Club 44: 509. 1917.

*Uredo Crotalariae* Dietel, Hedwigia 38: 256. 1899.

*Crotalaria anagyroides* H.B.K. Hacienda "Anacuri," Nor Yungas, Bolivia, June 5, 1920, 718.

Known otherwise only from two collections made in Brazil.

128. **Phakopsora Psoraleae** Jackson & Holway, sp. nov.

II. Uredinia hypophylloous, deep seated, thickly scattered, small in section, 135–180  $\mu$  broad, 120–150  $\mu$  high, at first punctiform, pale, opening by a pore; paraphyses cylindrical, clavate or somewhat capitate, occurring scattered throughout the spore bearing surface, more numerous at sides and abundant at apex where they are incurved; wall colorless thin, becoming tinted

around opening, thickened to  $6\ \mu$  or occasionally solid at apex; urediniospores short stipitate broadly ellipsoid,  $15-20\ \mu$  by  $24-30\ \mu$ , wall thin  $1.5\ \mu$ , finely and closely verrucose, the pores obscure, apparently several in an equatorial zone.

III. Teliospores not seen.

*Psoralea glandulosa* L. Sorata, Bolivia, Apr. 14, 1920,  
521 (type).

*Psoralea lasiostachya* Vog. Sorata, Bolivia, Apr. 22, 1920,  
566.

Though teliospores have not been seen we have little hesitation in assigning this *Uredo* to *Phakopsora*. The sori cover the leaves so uniformly as to suggest that the infection may possibly be systemic.

**Allopuccinia** Jackson, gen. nov.

Pycnia subcuticular, without ostiolar filaments. Aecia urediniod, surrounded by numerous paraphyses, urediniospores stipitate, teliospores stipitate, two-celled with colorless, thin walls, germinating at once.

129. **Allopuccinia diluta** Jackson & Holway, sp. nov.

O. Pycnia epiphyllous or occasionally hypophyllous, small, punctiform, closely gregarious in small groups above or among the uredinia or telia; subcuticular, hemispheric or conical in section,  $40-50\ \mu$  high,  $60-90\ \mu$  broad, ostiolar filaments none.

II. Uredinia hypophyllous, small  $0.2-0.5$  mm. across, golden or cinnamon-brown, scattered or gregarious, soon naked, ruptured epidermis not noticeable; paraphyses abundant, conspicuous, chiefly peripheral, variable, irregularly cylindrical, clavate or arcuate-clavate,  $40-65\ \mu$  long,  $6-15\ \mu$  wide; wall colorless or golden-brown, thin  $1-1\frac{1}{2}\ \mu$  except at apex in clavate forms, and on inner side of arcuate forms, irregularly thickened at apex or on one side  $3-6\ \mu$ ; urediniospores globoid or broadly ellipsoid  $15-18\ \mu$  by  $18-22\ \mu$ , finely and closely echinulate verrucose; wall thin  $1\frac{1}{2}-2\ \mu$ , pores obscure.

III. Telia like the uredinia, occasionally larger  $0.8-1$  mm. in diameter, and lighter in color due to abundant germination of spores, paraphyses as in uredinia; teliospores cylindrical or narrowly ellipsoid,  $14-18\ \mu$  by  $40-60\ \mu$ , rounded to obtuse above, rounded or narrowed to pedicel below, slightly or not constricted at the septum; wall thin  $1\ \mu$  or less, colorless, smooth, pores little differentiated, germinating at once from apex of

upper cell and at septum in lower cell; pedicel colorless, stout, equalling the spore or more commonly shorter.

*Amicia lobbiana* Benth. San Felipe, Prov. Sur Yungas, Bolivia, May 19, 1920, 611 (type); Sorata, Bolivia, Apr. 19, 1920, 549.

This species is made the type of a proposed new genus intended to provide for those species which would ordinarily be included in *Puccinia* but which, because of subcuticular pycnia (FIG. 4)



FIG. 4. Pycnia of *Allopuccinia diluta*.

and occurrence on Leguminosae, show relation to the Ravenelieae. The reasons for erecting this genus are the same as given in the discussion under *Mimema Holwayi* (page 339).

The pycnia in the above species are clearly subcuticular (FIG. 4) and of very different type than is characteristic of *Puccinia*. The species appears to be closely related to *P. Bergii* Speg., which was originally described as on *Adesmia punctata*, but the host identification has recently been corrected by Spegazzini (Rev. Argent. Bot. 1: 108. 1925), who now finds the host to be *Poiretia psoraloides*. This host genus is very closely related to *Amicia*. In the description of *P. Bergii*, however, no mention is made of paraphyses which form a very prominent character for the present species (FIG. 5).

The pycnia in this species appear to be associated with either uredinia or telia, or it may be that teliospores follow the urediniospores in the primary sorus.

Few species of *Puccinia* have been described on Leguminosae. *P. Piptadeniae* will probably prove to be a *Diorchidium*. In the

other species pycnia are unknown. Judging from the descriptions, however, it would seem entirely possible that the relation of these may prove to be with the above.

The teliospores germinate at once and have thin, colorless



FIG. 5. Uredinal sorus showing paraphyses of *Allopuccinia diluta*.

walls (FIG. 6). There appear to be no evident germ pores visible previous to germination. A comparison of figure 6 with figure 2 is suggestive of the close relationship of this form with *Mimema Holwayi*. The uredinia of the two species are also similar in that both have prominent and abundant incurved paraphyses.

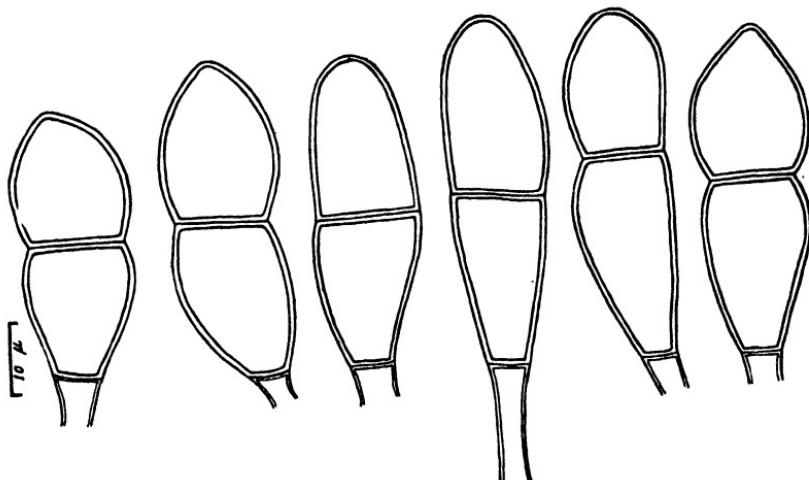


FIG. 6. Teliospores of *Allopuccinia diluta*. Note absence of pores. Compare with figure 2.

130. PUCCINIA OFFUSCATA Arth. Bull. Torrey Club **47**: 469.  
1920.

*Uredo Zorniae* Dietel, Hedwigia **38**: 257. 1899 (Not  
*P. Zorniae* McAlpine. 1906).

*Zornia diphylla* (L.) Pers. Cochabamba, Bolivia, March  
5, 1920, 370.

This species suggests relationship with the preceding. Pycnia are unknown for the species and do not appear to be present in the above collection.

131. RAVENELIA INDIGOFERAE Tranz. Hedwigia **33**: 369. 1894.

*Uredo Anilis* P. Henn. Hedwigia Beibl. **38**: 68. 1899.

*Pleoravenelia Indigoferae* Long, Bot. Gaz. **35**: 129. 1903.

*Indigofera suffruticosa* Mill. Nictheroy, Rio de Janeiro, Brazil, Aug. 22, 1921, 1060; Jacarépaguá, Rio de Janeiro, Brazil, Sept. 4, 1921, 1087; Bello Horizonte, Minas Geraes, Brazil, Dec. 1, 1921, 1355.

The collections listed above consist of uredinia only. The species is known in South America only from Brazil and Trinidad. It is also reported from Mexico, Guatemala, Cuba, Jamaica, and Bermuda.

132. RAVENELIA LONCHOCARPI Lagerh. & Dietel, Hedwigia **33**:  
46. 1894.

*Lonchocarpus* sp. São João, São Paulo, Brazil, March 19, 1922, 1655; Lapa, São Paulo, Brazil, June 4, 1922, 1941.

Known in South America otherwise only from Minas Geraes, Brazil. It has, however, been reported also from Cuba and Salvador.

133. *Uredo emendata* Jackson & Holway, sp. nov.

II. Uredinia hypophylloous, scattered, round 0.2–0.3 mm. across, soon naked, pulverulent, chestnut-brown, ruptured epidermis not conspicuous; paraphyses numerous, clavate or cylindric 12–18 by 30–75  $\mu$ , only slightly incurved, wall thin colorless, slightly or not at all thickened at apex or on one side;

urediniospores globoid, 18–22  $\mu$ , wall chestnut-brown 2–2½  $\mu$  thick, very finely and closely verrucose-echinulate, the pores 4, approximately equatorial.

*Meibomia Scorpiurus* (Sw.) Kuntze. Guayaquil, Ecuador,  
Aug. 1, 1920, 805.

134. *Uredo Eriosemae* Jackson & Holway, sp. nov.

II. *Uredinia* amphigenous, chiefly hypophyllous, minute, 0.2–0.3 mm. across, chestnut-brown, soon naked, pulverulent, ruptured epidermis conspicuous; paraphyses none; urediniospores globoid or broadly ellipsoid, 17–20  $\mu$  by 18–22  $\mu$ ; wall chestnut-brown 2–2½  $\mu$  thick, finely and moderately echinulate; pores 3 or 4 approximately equatorial.

*Eriosema crinitum* (H.B.K.) DC. Santa Anna, São Paulo, Brazil, May 28, 1922, 1902.

135. *UREDO SOLITARIA* Dietel & Neger in Engl. Bot. Jahrb. 27:  
16. 1899.

*Adesmia elegans* Clos. Panimavida, Chile, Dec. 20, 1919,  
245.

*Adesmia laxa* Clos. Termas de Chillan, Chile, Dec. 28,  
1919, 252.

Known otherwise only from the type collection made also in Chile.

136. *UROMYCES APPENDICULATUS* (Pers.) Link, Ges. Nat.  
Freunde Berlin Mag. 7: 28. 1815.

*Uredo appendiculata* Pers. Ann. Bot. Usteri 15: 16. 1795.

*Uredo Pamparum* Speg. Anal. Soc. Ci. Argent. 12: 74.  
1881.

*Uredo rufa* Speg. Anal. Soc. Ci. Argent. 17: 124. 1884.

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*Phaseolus vestitus* Hook. Chosica, Peru, July 23, 1920,  
783 1/2.

*Phaseolus* sp. Cochabamba, Bolivia, March 14, 1920, 411;  
Sorata, Bolivia, Apr. 15, 1920, 526; Nova Friburgo,  
Rio de Janeiro, Brazil, Jan. 7, 1922, 1465.

The first listed collection has dark thick-walled urediniospores. Telia are not present and it is referred to the above species provisionally.

**137. Uromyces Bradburyae Jackson & Holway, sp. nov.**

II. Uredinia amphigenous, scattered or gregarious, small round 0.3–0.5 mm. across, light chestnut-brown, somewhat tardily naked, pulverulent, ruptured epidermis conspicuous; urediniospores oblong or ellipsoid, somewhat flattened laterally, 18–24  $\mu$  by 23–28  $\mu$ ; wall cinnamon-brown or light chestnut-brown, 1½–2  $\mu$  thick, slightly thickened above and below, strongly and moderately echinulate, pores 2 equatorial.

III. Telia dark chestnut-brown, otherwise as in the uredinia; teliospores somewhat irregularly ellipsoid, ovoid, or obovoid, 16–24  $\mu$  by 27–36  $\mu$ ; wall light chestnut-brown, 2–3  $\mu$  thick, apex with an abrupt paler umbo 5–7  $\mu$  thick, appearing smooth but obscurely and evenly verrucose; pedicel colorless, collapsing, equalling the spore or shorter.

*Bradburya virginiana* (L.) Kuntze. Campos do Jordão,  
Brazil, May 2, 1922, 1801.

*Bradburya pubescens* (Benth.) Kuntze. Campos do Jordão,  
Brazil, April 20, 1922, 1735.

**138. UROMYCES CASTANEUS Sydow, Monog. Ured. 2: 94. 1909.**

*Desmodium incanum* Sw. Copacabana, Rio de Janeiro,  
Brazil, Sept. 21, 1921, 1136.

*Desmodium* sp. San Francisco, Nictheroy, Rio de Janeiro,  
Brazil, Sept. 23, 1921, 1146.

This distinct species is otherwise known only from the type collection on *D. incanum* made also at Rio de Janeiro, July 1887, by E. Ule (No. 664). A portion of the type collection has been seen.

**139. UROMYCES ELATUS Sydow, Ann. Myc. 6: 482. 1908.**

*Lupinus paniculatus* Desv. La Paz, Bolivia, March 18,  
1920, 417; May 14, 1920, 603; Cuzco, Peru, June 29,  
1920, 738.

*Lupinus soratensis* Rusby. Sorata, Bolivia, Apr. 22, 1920,  
561.

*Lupinus* sp. La Paz, Bolivia, March 24, 1920, 454;  
March 25, 1920, 459; May 12, 1920, 595, 596.

This -opsis form is easily distinguished by the conspicuous membranous peridium of theaecium and the long narrow colorless teliospores, germinating at once. The type collection

was made at La Paz, Bolivia, and has been previously reported from Peru by Arthur (Bot. Gaz. 65: 466. 1918).

140. *UROMYCES FABAE* (Pers.) deBary, Ann. Sci. Nat. IV. 20: 80. 1863.

*Uredo Fabae* Pers. Neues. Mag. Bot. 1: 93. 1794.

*Nigredo Fabae* Arth. N. Am. Fl. 7: 251. 1912.

*Vicia Faba* L. San Felipe, Chile, Sept. 25, 1919, 72; Concepción, Chile, Oct. 25, 1919, 138; La Paz, Bolivia, March 23, 1920, 443; Huigra, Chimborazo, Ecuador, Aug. 4, 1920, 838.

This species is evidently a common one in South America on this host, having been previously reported from Brazil, Argentina, Ecuador, and elsewhere. The rust is a cosmopolitan species.

141. *UROMYCES FLECTENS* Lagerh. Sv. Bot. Tidskr. 3: 36. 1909.

*Trifolium repens* L. Valdivia, Chile, Nov. 13, 1919, 174; Cochabamba, Bolivia, March 1, 1920, 353; Quito, Ecuador, Aug. 13, 1920, 882; Cuenca, Ecuador, Sept. 13, 1920, 984.

All the collections from South America which we have seen include telia only, and appear to be microcyclic, though no pycnia have been observed. This is the form described by Lagerheim (l.c.). On *Trifolium repens* there is a series of forms of different life cycle but with similar morphology. The long cycle form, with all spore forms is either referred to *U. Trifolii* Lev. or to *U. Trifolii-repentis* (Cast.) Liro. The -opsis form is *Uromyces nerviphila* (Grognot). Transitional forms occur particularly in the -opsis form. One may find in the same collection groups consisting of pycnia and aecia or pycnia and telia.

142. *UROMYCES HEDYSARI-PANICULATI* (Schw.) Ellis, N. Am. Fungi 246. 1878.

*Puccinia Hedysari-paniculati* Schw. Schr. Nat. Ges. Leipzig 1: 74. 1822.

*Uredo Desmodii-leiocarpi* P. Henn. Hedwigia 41: 107. 1902.

*Uromyces Desmodii-leiocarpi* P. Henn. Hedwigia 48: 1. 1908.

*Meibomia* sp. Cochabamba, Bolivia, March 4, 1920, 364; Hacienda Anacuri, Prov. Nor Yungas, Bolivia, June 4, 1920, 710; Huigra, Chimborazo, Ecuador, Aug. 7, 1920, 857; Rio de Janeiro, Brazil, Aug. 29, 1921, 1079; Copacabana, Rio de Janeiro, Brazil, Sept. 21, 1921, 1135; Alto da Serra, São Paulo, Brazil, Jan. 28, 1922, 1506; Juquery, São Paulo, Brazil, Feb. 14, 1922, 1555; Cantareira, São Paulo, Brazil, Feb. 18, 1922, 1566, 1567; São Roque, São Paulo, Brazil, March 21, 1922, 1666; Campos do Jordão, São Paulo, Brazil, Apr. 25, 1922, 1769.

143. *UROMYCES LATIYRINUS* Speg. Anal. Soc. Ci. Argent. **12**: 71. 1881.

*Aecidium lathyrinus* Speg. Anal. Soc. Ci. Argent. **12**: 33. 1881.

*Uromyces clavatus* Diet. Hedwigia **36**: 27. 1897.

*Uromyces Chilensis* Dietel & Neger in Engler, Bot. Jahrb. **24**: 154. 1897.

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*Lathyrus magellanicus* Lam. San José de Maipo, Chile, Oct. 8, 1919, 97; Constitucion, Chile, Oct. 15, 1919, 118.

144. *UROMYCES MEDICAGINIS* Pass. in Thüm. Herb. Myc. Oecon. **156**. 1874.

*Uredo medicaginicola* Speg. Anal. Mus. Nac. Buenos Aires **6**: 234. 1898.

*Nigredo Medicaginis* Arth. N. Am. Fl. **7**: 256. 1912.

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*Medicago Sativa* L. Cordoba, Argentina, Aug. 11, 1922, 2021.

This species, common in North America and Europe, has been reported from South America only from Argentina and southern Brazil.

145. *UROMYCES NEUROCARPI* Dietel, Hedwigia **34**: 292. 1895.

*Uromyces rostratus* P. Henn. Hedwigia **35**: 227. 1896.

*Uromyces insularis* Arth. Bull. Torrey Club **33**: 515. 1906.

*Nigredo Neurocarpi* Arth. N. Am. Fl. 7: 258. 1912.

*Clitoria brachystegia* Benth. Portovelo, Prov. de Oro, Ecuador, Sept. 22, 1920, 1000.

*Clitoria cajanifolia* Benth. Jacarépaguá, Rio de Janeiro, Brazil, Sept. 4, 1921, 1088; Nov. 16, 1921, 1314; Copacabana, Rio de Janeiro, Brazil, Sept. 21, 1921, 1138.

A characteristic species, easily recognized by the thin-walled, elongated teliospores which germinate at once. *U. rostratus* P. Henn. was originally described as on *Eriosema*, an error for *Clitoria*, as pointed out by Dietel and Sydow. The species is reported from South America only from Brazil. It is also known from Trinidad, Porto Rico, Cuba, and Jamaica in the West Indies, and in Central America from Salvador.

146. *UROMYCES ORBICULARIS* Diet. Hedwigia 36: 28. 1897.

*Desmodium adscendens* DC. Sorata, Bolivia, Apr. 12, 1920, 505.

A very distinct -opsis form known previously only from the type collection made in Brazil. The aecia are hypophylloous, have prominent cylindrical peridia and the telia develop opposite them on the upper surface, evidently from the same mycelium.

147. *UROMYCES TENUISTIPES* Dietel & Holw.; Holway, Bot. Gaz. 24: 25. 1897.

*Desmodium uncinatum* Jacq. Cochabamba, Bolivia, March 14, 1920, 408.

*Desmodium* sp. Quito, Ecuador, Aug. 21, 1920, 931.

This characteristic species is known otherwise only from Mexico.

148. *UROMYCES TRIFOLII* (Hedw. f.) Lév. Ann. Sci. Nat. III. 8: 371. 1847.

*Puccinia Trifolii* Hedw. f. DC. Fl. Fr. 2: 225. 1805.

*Trifolium amabile* H.B.K. La Paz, Bolivia, March 19, 1920, 426, March 30, 1920, 485; Sorata, Bolivia, Apr. 27, 1920, 577.

*Trifolium* sp. Cochabamba, Bolivia, March 1, 1920, 352.

Aecia are abundant in collections 485 and 577. The rust resembles the long cycled form so common in North America on *Trifolium repens* L.

149. **Uromyces Trifolii-megalanthi** (Ditel & Neger) Jackson & Holway, comb. nov.

*Aecidium Trifolii-megalanthi* Dietel & Neger in Engler, Bot. Jahrb. 27: 14. 1899.

*Trifolium* sp. Papudo, Chile, Sept. 18, 1919, 45; Concepción, Chile, Oct. 29, 1919, 149.

This species, originally described from Chile in the aecial form only, appears to be distinct. Both the collections listed above include telia. It is apparently related to *U. oblongus* Vize and to *U. elegans* (Berk.) Lag. The telia are small, round, pulverulent, somewhat tardily naked, blackish-brown. The teliospores are globoid or broadly ellipsoid, 18–22  $\mu$  by 20–24  $\mu$ , rounded above and below, wall evenly 2.5–3  $\mu$  thick with an inconspicuous hyaline papilla over the pore. The wall at first appears to be smooth but there are inconspicuous colorless markings sparsely placed along one to three longitudinal lines.

The species has more nearly the spore character of *U. oblongus* with the sorus character of *U. elegans*.

150. **UROMYCES VIGNAE** Barclay, Jour. Asiatic Soc. Bengal 60: 211. 1891.

*Vigna luteola* (L.) Benth. Guayaquil, Ecuador, July 31, 1920, 803.

This species, according to Fromme (Phytopathology 14: 67–79, 1924) is distinguished from *U. appendiculata* primarily in the superequatorial position of the pores in the urediniospores.

151. **Uropyxis Amiciae** (Vesterg.) Jackson & Holway, comb. nov.

*Puccinia Amiciae* Vesterg. Micro. rar. Sel. 1363, 1909 (nomen nudum).

II. Uredinia amphigenous, round, 0.3–0.8 mm. across, early naked, golden-brown, appearing compact due to abundant paraphyses, ruptured epidermis not conspicuous; paraphyses numerous, conspicuous, variable, chiefly periferous and arcuate-

clavate, 8–18  $\mu$  by 45–75  $\mu$ , wall colorless or tinted golden-brown, thin on the inside, thickened 4–6  $\mu$  on the outer side, often solidly thickened at apex; urediniospores globoid or broadly ellipsoid, 15–18  $\mu$  by 16–20  $\mu$ ; wall colorless or tinted golden-brown, 3  $\mu$  thick, closely and prominently echinulate, pores numerous, scattered.

III. Telia like the uredinia, blackish-brown; teliospores ellipsoid, regular, 18–20  $\mu$  by 28–34  $\mu$ , inner wall 1½–2½  $\mu$  thick, chestnut-brown, outer wall colorless, 3  $\mu$  thick adorned by inconspicuous verrucose thickenings placed about 3  $\mu$  apart; pedicel colorless usually deciduous.

*Amicia parvula* Rusby. Cochabamba, Bolivia, March 7, 1920, 375.

152. *UROPYXIS DALEAE* (Ditel & Holw.) Magn. Ber. Deuts. Bot. Ges. 17: 115. 1899.

*Puccinia Daleae* Dietel & Holw. Bot. Gaz. 24: 27. 1897.

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*Purosela pazensis* Rusby. Cochabamba, Bolivia, Feb. 26, March 11, 1920, 333, 401.

This species has not been previously reported from South America. It is common in Mexico and reported from Guatemala and Salvador in Central America.

#### SPECIES ON GERANIACEAE

153. *Puccinia calliquensis* Neger, Anal. Univ. Chile 93: 777. 1896.

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*Geranium Berterianum* Colla. Termas de Chillan, Chile, Dec. 31, 1919, 262.

This species and the next following have a very similar habit. *P. calliquensis*, however, differs in the thickness of the teliospore wall which is much thinner, 2  $\mu$  or less, and in the markings which are uniformly finely verrucose. The species has evidently been reported previously only on the above host and only from the type locality also in Chile.

154. *Puccinia distenta* Jackson & Holway, sp. nov.

II. Uredinia hypophylloous, scattered or gregarious, round, 0.5–1 mm. across, dark, cinnamon-brown, somewhat tardily naked, pulverulent or plane, ruptured epidermis at first con-

spicuous later disappearing; urediniospores ellipsoid or obovoid, 22–26  $\mu$  by 28–30  $\mu$ ; wall light cinnamon-brown 2–3  $\mu$  thick, evenly, minutely and rather sparsely echinulate, the pores 2 equatorial or approximately so, covered by a hyaline membrane which swells considerably in water.

III. Telia like the uredinia, blackish; teliospores more commonly found in the uredinia, ellipsoid or obovoid, rounded above and below, 26–32  $\mu$  by 38–46  $\mu$ , slightly or not at all constricted at septum; wall chestnut-brown, smooth, 3–5  $\mu$  thick, thickened to 9  $\mu$  at apex; pedicel short, colorless, deciduous.

*Geranium Ochensis* Phil. Viña del Mar, Chile, Sept. 10, 1919,  
19 (type).

*Geranium Core-Core* Steud. Constitucion, Chile, Oct. 17,  
1919, 123.

*Geranium* sp. Sorata, Bolivia, Apr. 27, 1920, 576; La Falda, Argentina, Aug. 14, 1922, 2027.

The urediniospores of this species are similar to the preceding, but the teliospores differ markedly, in their lack of a conspicuous constriction at the septum and in the smooth walls. The species is evidently an eu-form as old aecia, too imperfect for adequate description, are present on one leaf.

155. PUCCINIA LEVEILLEI Mont. in C. Gay. Fl. Chil. 8: 41.  
1852.

*Puccinia Geranii* Lév. Ann. Sci. Nat. III. 5: 270. 1846.  
Not *P. Geranii* Corda. 1840.

*Puccinia Geranii-sylvatici* P. Karst. Not Sällsk. Faun.  
Fl. Fenn. 8: 220. 1869.

*Puccinia Leveilleana* DeToni in Sacc. Syll. Fung. 7: 696.  
1888.

*Micropuccinia Leveillei* Arth. & Jackson; Arth. Bull.  
Torrey Club 48: 41. 1921.

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*Geranium Berterianum* Colla. Termas de Chillan, Chile,  
Dec. 31, 1919, 261.

*Geranium* sp. Cochabamba, Bolivia, March 11, 1920,  
400; La Paz, Bolivia, March 24, 1920, 458.

This characteristic micro-form was originally described from Chile and has apparently not been previously reported elsewhere in South America, unless *P. geranicola* Speg. from Patagonia

should prove synonymous. The species is, however, not infrequent in western North America, also in Europe.

156. UREDO UNILATERALIS Arth. Bull. Torrey Club **45**: 155.  
1918.

*Geranium chilloënsse* Willd. Quito, Ecuador, Aug. 15, 1920,  
895.

*Geranium sodiroanum* Kunth. Quito, Ecuador, Aug. 15,  
1920, 902.

This characteristic species has not previously been reported from South America. It is otherwise known only from the type locality in Mexico. The spathulate-obovoid spores, flattened on one side and with a single germ pore on the flattened side serve to distinguish this species from all others on *Geranium*.

#### SPECIES ON OXALIDACEAE

157. PUCCINIA OXALIDIS (Lév.) Dietel & Ellis; Dietel, Hedwigia  
**34**: 291. 1895.

*Uredo Oxalidis* Lév. Ann. Sci. Nat. II. **16**: 240. 1841.

*Aecidium mexicanum* Maubl. Bull. Soc. Myc. Fr. **20**: 73.  
1904. Not *A. mexicanum* Dietel & Holway. 1897.

*Aecidium Maublancii* Sydow, Monog. Ured. **2**: 351. 1904.

*Oxalis scandens* H.B.K. Sorata, Bolivia, Apr. 27, 1920,  
580; Quito, Ecuador, Aug. 31, 1920, 956.

*Oxalis* sp. Sorata, Bolivia, Apr. 12, 1920, 509, 510;  
San Felipe, Prov. Sur Yungas, Bolivia, May 19,  
1920, 614; Quito, Ecuador, Aug. 28, 1920, 950;  
Silvestre, Rio de Janeiro, Brazil, Sept. 16, 1921,  
1118; Guarulhos, São Paulo, Brazil, Jan. 30, 1922,  
1514.

This species is evidently a common one throughout South America and the West Indies. It is also known from Mexico and the southern United States. The rust is heteroecious with aecia on *Mahonia*. No collection of aecia has as yet been reported from South America.

## SPECIES ON ERYTHROXYLACEAE

158. *UREDO ERYTHROXYLONIS* Graz. Bull. Soc. Myc. Fr. 7: 152.  
1891.

*Erythroxylon Coca* Lam. Hacienda Anacuri, Nor Yungas,  
Bolivia, June 3, 1920, 707.

This is the only rust known on this host family in South America. The species was originally described from material collected in Bolivia and Peru. It has also been reported from Venezuela and Brazil and is known to occur in Cuba and Porto Rico. It apparently occurs wherever coca is cultivated.

## SPECIES ON MALPIGHIACEAE

159. *Aecidium vinnulum* Jackson & Holway, sp. nov.

O. Pycnia epiphyllous, grouped in the center of spots opposite the aecia; punctiform, conspicuous, subcuticular or intraepidermal, applanate, or broadly conical,  $160 \times 215 \mu$  in diameter,  $60-100 \mu$  high, ostiolar filaments converging, not conspicuous.

I. Aecia hypophyllous in groups on discolored spots 8-12 mm. across, numerous, cupulate, small; peridia white reflexed becoming fimbriate, peridial cells rhomboid in side view, considerably overlapping,  $28-36 \times 14-16 \mu$ , outer wall smooth,  $5-6 \mu$  thick, inner wall finely verrucose,  $3-5 \mu$  thick; aeciospores somewhat angular, broadly ellipsoid,  $16-18 \times 20-24 \mu$ , wall thin,  $1 \mu$  or less, finely and inconspicuously verrucose.

*Byrsonima intermedia* Juss. Guarulhos, São Paulo, Brazil,  
Jan. 30, 1922, 1511.

This *Aecidium* appears to differ markedly from any previously described on this host and family. The pycnia are not subepidermal, but rather intraepidermal. The epidermal cells are quite large in this host species and the pycnia appear to develop at first between the cells, later digesting the upper portion, and finally are covered by the outer epidermal wall and the cuticle. The character of the pycnia suggests that this aecidium is connected with a rust belonging to the Melampsoraceae rather than the Pucciniaceae. Is it possible that this *Aecidium* is the aecial stage of *Crossopsora*? It will be noted that we have assigned tentatively two collections of *Uredo* to *Crossopsora notata* collected a few weeks later in the same region and on the same host.

160. *CROSSOPSORA NOTATA* Arth. N. Am. Fl. 7: 695. 1925.*Uredo notata* Arth. Mycologia 9: 89. 1917.*Cronartium notatum* Arth. Mem. Torrey Club 17: 114. 1918.*Byrsonima crassifolia* H.B.K. Hacienda Anacuri, Prov. Nor Yungas, Bolivia, June 4, 1920, II, III, 717.*Byrsonima intermedia* Juss. São Paulo, Brazil, Feb. 15, 1922, II, 1560; Mandaqui, São Paulo, Brazil, May 25, 1922, II, 1886.

So far as we are aware, this is the first record of this species in South America. It was described originally from Porto Rico and is also known to occur in Cuba.

161. *PUCCINIA HETEROPTERIDIS* Thüm. Myc. Univ. 839, 1877.*Heteropteris* sp. Villa Prudente, São Paulo, Brazil, May 31, 1922, 1926; Guarulhos, São Paulo, Brazil, June 1, 1922, 1934; Guapira, São Paulo, Brazil, June 11, 1922, 1957.

An apparently distinct species, reported originally from Uruguay. It has been collected a number of times previously in Argentina and Brazil.

162. *PUCCINIA INFLATA* Arth. Bull. Torrey Club 33: 516. 1906.*Bullaria inflata* Arth. & Mains, N. Am. Fl. 7: 486. 1922.

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*Stigmaphylon* sp. São João, São Paulo, Brazil, July 2, 1922, 1994.

This collection was identified as above as it appears to fit that species admirably. It seems entirely probable, however, that this species is not distinct from the *Puccinia insueta* Wint. What appears to be needed before species limits can be satisfactorily established in this group of rusts is to have available an abundant series of specimens, the hosts for which have been accurately identified specifically. With the host situation as it is at present one obtains the impression that there is one very variable species, which may or may not be the true interpretation.

163. *Puccinia inrecta* Jackson & Holway, sp. nov.

II. Uredinia amphigenous, small, round, 0.3–0.5 mm. in diameter, cinnamon brown, early naked, pulverulent, ruptured

epidermis conspicuous; urediniospores ellipsoid or obovoid, 20–24 by 28–34  $\mu$ ; wall cinnamon brown, 1.5–2.5  $\mu$  thick, sparsely, prominently and rather sharply echinulate; pores 4, approximately equatorial or more commonly scattered.

III. Telia like the uredinia, more commonly epiphyllous, blackish brown; teliospores somewhat irregularly ellipsoid, 25–29 by 32–38  $\mu$ , slightly constricted; wall chestnut brown, 2.5–3  $\mu$  thick, slightly thickened, 4.5  $\mu$  over pores, evenly and closely rugose-reticulate, the depressions often arranged in longitudinal lines; pedicel colorless, usually attached on one side of lower cell or near septum, apex swelling considerably in water, usually deciduous below the swelling.

*Banisteria campestris* Juss. Jardin d'Acclimacão, São Paulo, Brazil, Jan. 23, 1922, 1496; April 15, 1922, 1734 (type).

This species appears to be quite different from *P. Banisteriae* P. Henn., which has the pedicel attached at the base and the wall markings described as verrucose. Our species may be the same as *Uredo banistericola* P. Henn. which is reported from the same region and answers our description quite well. Material has, however, not been available for comparison.

This species shows relationship to *P. insueta* and others, but seems to be amply distinct. The uredospores do not show the peculiar thickened walls of that species and the echinulate markings, while of the same type, are closer set and not so prominent.

164. PUCCINIA INSUETA Wint. *Hedwigia* 26: 27. 1887.

*Diorchidium insuetum* Magnus, Ber. Deutsch. Bot. Ges. 9: 192. 1891.

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*Stigmaphylon acuminatum* Juss. Nictheroy, Rio de Janeiro, Brazil, Aug. 18, 1921, 1051.

*Stigmaphylon affine* Juss. Juquery, São Paulo, Brazil, Feb. 2, 1922, 1529; Hacienda, La Florida, Prov. Sur Yungas, Bolivia, May 27, 1920, 668.

*Stigmaphylon tomentosum* Juss. Cascadura, Rio de Janeiro, Brazil, Aug. 24, 1921, 1067.

*Stigmaphylon vitifolium* St. Hill. Nictheroy, Rio de Janeiro, Brazil, Aug. 18, 1921, 1050; Nov. 15, 1921,

1305; Bom Successo, Rio de Janeiro, Sept. 13, 1921,  
1108.

*Stigmaphyllum* sp. Fonseca, Nictheroy, Rio de Janeiro, Brazil, Sept. 18, 1921, 1124; Freguesia, Rio de Janeiro, Brazil, Nov. 18, 1921, 1317; Friburgo, Rio de Janeiro, Brazil, Jan. 6, 1922, 1461; Lapa, São Paulo, Brazil, June 4, 1922, 1939; Petropolis, Rio de Janeiro, Brazil, Oct. 20, 1921, 1232, Oct. 30, 1921, 1257; Prata, São Paulo, Brazil, Apr. 7, 1922, 1703; Silvestré, Rio de Janeiro, Brazil, Dec. 25, 1921, 1424.

As here interpreted this is a variable species which on further study may include more than one closely related species. In general we have included those forms in which the outer wall of the urediniospore is colorless, swelling considerably and with prominent but remote echinulate markings. The teliospores are also variable in the different collections, both as to the character of the rugose markings and the size of the swelling at apex of the pedicel and the position of insertion of the latter.

The last four collections listed have smaller urediniospores and it is somewhat doubtful whether the host is *Stigmaphyllum*.

#### 165. *Puccinia picturata* Jackson & Holway, sp. nov.

II. Uredinia amphigenous, chiefly hypophyllous, scattered, cinnamon brown, round, 0.5–0.8 mm. across, tardily naked, pulv erulent, ruptured epidermis conspicuous; urediniospores broadly ellipsoid or obovoid approaching globoid, 32–40 × 35–45  $\mu$ ; wall thick, 6–9  $\mu$ , often gradually thickened toward apex to 12  $\mu$ , appearing to be made up of two layers, the outer nearly colorless, the inner golden brown, strongly, sparsely and sharply echinulate, the markings placed 6–8  $\mu$  apart and reaching a height of 2.5  $\mu$ ; pores obscure.

III. Telia like the uredinia, blackish; teliospores ellipsoid or oblong, 25–32 × 38–46  $\mu$ , rounded at either end, slightly or not at all constricted; wall opaque in water mount, appearing to be of two layers in lacto-phenol, the outer thin, slightly tinted golden brown, the inner thick, dull blackish brown, 3.5–5  $\mu$  thick, slightly thickened to 7.5  $\mu$  on side occupied by pores, noticeably rugose-reticulate by the anastomosing of ridges having an uneven edge, especially in the upper part of spore, tending to be smooth on the side to which the pedicel is attached; pedicel colorless, below, inflated to a depressed globoid sack 20–25  $\mu$  wide at point

of attachment which is usually on one side near the septum, the wall on the side of swelling next the spore often slightly tinted brownish, pedicel usually deciduous at lower side of inflation.

*Heteropteris*? Juiz de Fora, Rio de Janeiro, Brazil, Dec. 17, 1921, 1401.

An interesting species showing relation to *Puccinia insueta* Winter and *P. inflata* Arth., but apparently distinct. The ure-diniospores are larger and the teliospore color and markings appear very distinct in comparative study.

These species are difficult to describe, the inflated pedicel is attached at one side and the two cells of the spore are borne in a semihorizontal position. The pores, one in each cell, occur on the upper side of the spore in relation to the point of attachment of the pedicel. The thickness of the wall is greater around the pores and the markings are more prominent on the upper side.

#### SPECIES ON RUTACEAE

166. AECIDIUM RICKII Sydow, Monog. Ured. 4: 201. 1923.

*Xanthoxylum* sp. Therezopolis, Rio de Janeiro, Brazil, Oct. 2, 1921, 1187.

A very distinct species easily distinguished from the following by the larger size of the aeciospores with thickened apical walls. Otherwise known only from the type collection, also from Brazil (Thiessen, Dec. fung. Bras. 191).

167. AECIDIUM XANTHOXYLINUM Speg. Rev. Argent. Hist. Nat. Buenos Aires 1: 400. 1891.

*Xanthoxylum* sp. Friburgo, Rio de Janeiro, Brazil, Jan. 7, 1922, 1468.

The type collection of this species was made in Paraguay. It has been doubtfully reported by Dietel from Brazil.

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## CERCOSPORA STUDIES—II. SOME TROPICAL CERCOSPORAE<sup>1</sup>

W. G. SOLHEIM AND F. L. STEVENS

(WITH 12 TEXT FIGURES)

This paper is the second in a series dealing with the genus *Cercospora*. In these studies the primary aims are to give adequate descriptions of the various species, to acquire definite concepts of individual species, and to assign the species to definite morphological groups within which comparisons may be made. No special effort is, therefore, being made at present to compare species. It is thought best to leave this until definite morphological groupings have been secured. Species are, therefore, reduced to synonymy only when the evidence is quite obvious.

In the first paper it was pointed out that conidia may germinate, producing conidiophores bearing secondary conidia. This has also been observed in several of the species herein described. In addition it has been observed that the conidia may produce secondary conidia without the production of conidiophores. When this is the case a secondary conidium is cut off from the tip of the conidium. Several more may be produced very near the tip. The primary conidium usually enlarges slightly at the tip and the secondary conidia are borne on short, dentate projections on the enlargement. This has been observed in *C. Cayaponiae* Stev. & Solh. and *C. Porophylli* Stev. & Moore.

It has been pointed out by various workers that certain fungi now recorded as *Cercospora* produce conidia catenately. This character seems to be worthy of generic distinction. A new genus, *Ragnhildiana*, is, therefore, proposed (p. 402) to contain all such forms.

In the first paper the morphological characters suggested as a basis for sectioning the genus were outlined. Among these was the presence or absence of an external mycelium. It appears,

<sup>1</sup>The first paper of this series was published by W. G. Solheim in Ill. Biol. Mon. 12: 1-84. 1929. (Issued March 7, 1930.)

after further study, that this factor can have little significance as a sectional character. Specimens of the same species have been seen with or without the external mycelium. The characteristic is of some value in species delimitation, however, as numerous cases occur in which there never is an external mycelium, while others always have it present.

Another character has been selected as having considerable value. This is the nature of the conidial scars, whether they are minute or indistinct or relatively large and distinct. The size of the scar appears to vary little no matter what the variation is in other characters. Using this character and eliminating the mycelial characteristic referred to above, the revised key to the sections is as follows:

#### KEY TO SECTIONS OF CERCOSPORA

- I. Conidial scars indistinct, or, if distinct, then  $2\ \mu$  or less in diameter.
  - A. Conidiophores simple.
    1. Stroma tuberculate.
      - a. Conidia acicular-obclavate..... Section I.
      - b. Conidia abruptly obclavate..... Section II.
      - c. Conidia cylindrical..... Section III.
    2. Stroma not tuberculate, composed of loosely to fairly compactly interwoven hyphae.
      - a. Conidia acicular-obclavate..... Section IV.
      - b. Conidia abruptly obclavate..... Section V.
      - c. Conidia cylindrical..... Section VI.
  - B. Conidiophores branched.
    1. Branching opposite..... Section VII.
    2. Branching alternate.
      - a. Stroma tuberculate.
        1. Conidia acicular-obclavate..... Section VIII.
        2. Conidia abruptly obclavate..... Section IX.
        3. Conidia cylindrical..... Section X.
      - b. Stroma not tuberculate, composed of loosely to fairly compactly interwoven hyphae.
        1. Conidia acicular-obclavate..... Section XI.
        2. Conidia abruptly obclavate..... Section XII.
        3. Conidia cylindrical..... Section XIII.
    3. Branching alternate and opposite.
      - a. Stroma tuberculate.
        1. Conidia acicular-obclavate..... Section XIV.
        2. Conidia abruptly obclavate..... Section XV.
        3. Conidia cylindrical..... Section XVI.

- b. Stroma not tuberculate, composed of loosely to fairly compactly interwoven hyphae.
  - 1. Conidia acicular-obclavate..... Section XVII.
  - 2. Conidia abruptly obclavate..... Section XVIII.
  - 3. Conidia cylindrical..... Section XIX.
- II. Conidial scars distinct, over  $2\ \mu$  in diameter.
  - A. Conidiophores simple.
    - 1. Stroma tuberculate.
      - a. Conidia acicular-obclavate..... Section XX.
      - b. Conidia abruptly obclavate..... Section XXI.
      - c. Conidia cylindrical..... Section XXII.
    - 2. Stroma not tuberculate, composed of loosely to fairly compactly interwoven hyphae.
      - a. Conidia acicular-obclavate..... Section Eucercospora.<sup>1</sup>
      - b. Conidia abruptly obclavate..... Section XXIV.
      - c. Conidia cylindrical..... Section XXV.
  - B. Conidiophores branched.
    - 1. Branching opposite..... Section XXVI.
    - 2. Branching alternate.
      - a. Stroma tuberculate.
        - 1. Conidia acicular-obclavate..... Section XXVII.
        - 2. Conidia abruptly obclavate..... Section XXVIII.
        - 3. Conidia cylindrical..... Section XXIX.
      - b. Stroma not tuberculate, composed of loosely to fairly compactly interwoven hyphae.
        - 1. Conidia acicular-obclavate..... Section XXX.
        - 2. Conidia abruptly obclavate..... Section XXXI.
        - 3. Conidia cylindrical..... Section XXXII.
    - 3. Branching alternate and opposite.
      - a. Stroma tuberculate.
        - 1. Conidia acicular-obclavate..... Section XXXIII.
        - 2. Conidia abruptly obclavate..... Section XXXIV.
        - 3. Conidia cylindrical..... Section XXXV.
      - b. Stroma not tuberculate, composed of loosely to fairly compactly interwoven hyphae.
        - 1. Conidia acicular-obclavate..... Section XXXVI.
        - 2. Conidia abruptly obclavate..... Section XXXVII.
        - 3. Conidia cylindrical..... Section XXXVIII.

## SECTION I

Conidial scars minute or indistinct, conidiophores simple, stroma tuberculate, conidia acicular-obclavate.

<sup>1</sup> It is the intention to name all the sections eventually.

CERCOSPORA RACIBORSKII Sacc. & Sydow, Syll. Fung. 16: 1070. 1902.

Type locality: Java.

Spots amphigenous, circular to somewhat angular, at times confluent, concentrically zoned, 1-15 mm., brown; border definite, raised, olivaceous to brown. Mycelium internal, subhyaline to olive-brown, 1.5-7  $\mu$ . Conidiophores amphigenous, solitary or loosely to moderately tufted, rupturing the epidermis, simple, straight, arising from a fairly compact to tuberculate stroma, olivaceous to brown with a reddish tinge, 35-140  $\times$  4-5.5  $\mu$ , continuous or 1-4-septate; conidial scars mostly indistinct, laterally displaced, rarely shouldered. Conidia acicular, apex rounded, subhyaline to olivaceous, 35-140  $\times$  2.5-3.5  $\times$  2-2.5  $\mu$ , 3-20-septate.

On leaves of *Nicotiana* sp. and \**Nicotiana Tabacum* L.

The specimen examined agrees well with the Saccardian description except that Saccardo records the conidia as being only 3-5-septate and 60-180  $\times$  4-4.5  $\mu$ . This difference may or may not be of any significance.

The above species is very closely related to *C. Nicotianae* Ellis & Ev. from which it differs in producing a decidedly different type of spot and in having conidia with rounded apices and being much more prominently septate.

Specimens examined: Herb. Univ. Ill., Hawaiian Fungi No. 925, Kealakekua.

## SECTION II

Conidial scars minute or indistinct, conidiophores simple, stroma tuberculate, conidia abruptly obclavate.

CERCOSPORA PERSONATA (Berk. & Curt.) Ellis & Ev. Jour. Myc. 1: 63. 1885.—Sacc. Syll. Fung. 4: 439. 1886.—Atkinson, Jour. Elisha Mitch. Soc. 8: 43. 1891.—P. Henn. Hedwigia 43: 395. 1904.—Heald & Wolf, Bur. Pl. Ind. Bull. 226: 49. 1912. Pl. 3, fig. 9.—Wolf, Ala. Exp. Sta. Bull. 180: 129-139. 1914. Pl. 1; Pl. 3, fig. 2; Pl. 5, figs. 1-2.—Kew Bull. 1920: 299.—Welles, Am. Jour. Bot. 12: 202. 1925. Pl. 18, figs. 45-46.

\* Starred names indicate hosts on which the fungus has been seen by the authors.

Syn. *Cladosporium personatum* Berk. & Curt. *Grevillea* 3: 106.  
1875.

*Cercospora Arachidis* P. Henn. *Hedwigia* 41: 18. 1902.  
—*Sacc. Syll. Fung.* 18: 600. 1906.

Type locality: Carolina.

Spots amphigenous, circular on leaf blade, 1–5 mm., oblong to elliptical on rachis and petiole, 1–2 × 4–6 mm., brown to blackish-brown, raised; border indefinite. Mycelium internal, possibly also external, subhyaline, 1–2.5  $\mu$ , stromatic mycelium brown, 2–5  $\mu$ . Conidiophores hypophyllous or occasionally also being produced above, very densely tufted, rupturing the epidermis or emerging through the stomata and rupturing the surrounding epidermis, erect, simple, somewhat geniculate above, tuberculate, brown with reddish tinge, 35–80 × 4–6.5  $\mu$ , continuous or 1–2-septate; conidial scars minute but distinct, aggregated towards tips, more or less laterally displaced and warty to shouldered. Conidia abruptly obclavate to cylindrical, pale-brown to oliveaceous to yellowish-brown, 25–65 × 5–7.5 × 3.5–5  $\mu$ , 2–8-septate.

On leaf blades, rachis and petioles of *\*Arachis hypogaea* L. and on leaves of *Cassia occidentalis* L. and *C. corymbosa* Lam.

Welles reports conidiophores up to 154.8  $\mu$  and conidia up to 184  $\mu$ .

Wolf has reported this species as producing spermogonia. These have been observed in several of the specimens examined.

The specimen collected by F. M. Bailey in 1913, Herb. Univ. of Ill., ex Herb. Hort. Bot. Reg. Kew, from Queensland, Australia, is not *C. personata*. Beyond this the material did not permit determination of the fungus present.

Specimens examined: Herb. Univ. Ill. No. 32983, ex Herb. U. S. Dept. Agric. No. 5032, Starkville, Miss.; No. 20078, ex Herb. S. M. Tracy, Starkville, Miss.; No. 5121, J. A. Stevenson, Rio Piedras, Porto Rico; F. L. Stevens, Porto Rican Fungi Nos. 2447 and 2506, Rio Piedras.—Ellis & Ev. N. Am. Fungi No. 2480, Starkville, Miss.

### SECTION III

Conidial scars minute or indistinct, conidiophores simple, stroma tuberculate, conidia cylindrical.

CERCOSPORA DEPAZEOIDES (Desm.) Sacc. *Fungi Veneti* 5: 187; *Fungi Ital.* Pl. 645; *Nuovo Gior. Bot. Ital.* 8: 187. 1876; *Sacc. Syll. Fung.* 4: 469. 1886.—Frank, *Krankh. Pfl.* p. 602. 1880.—Ellis & Ev. *Jour. Myc.* 1: 34. 1885.—Atkinson, *Jour. Elisha Mitch. Soc.* 8: 61. 1891.—*Malpighia* 14: 226. 1900.—Lindaw in Rabenh. *Krypt.-Fl.* 9: 134. 1910.

Syn. *Exosporium depazeoides* Desm. *Ann. Sci. Nat.* III. 11: 364. 1849.—Kickx, *Fl. Crypt. Fl.* II, 101.—Lamb. *Fl. Myc.* III, 198.

*Passalora penicillata* Cesati in Klotzsch, *Herb. Myc.* 2 ed. 587. 1857.

*Vermicularia depazeoides* Westend in *Prodr. Fl. Bot.* II, 4, p. 114. 1851.

*Cercospora penicillata* (Cesati) Fres. *Beitr.* 3: 93. 1863.

*Cercospora sambucina* Ellis & Kellerm. *Am. Nat.* 17: 1166. 1883.

*Cercospora Sambuci* Stev. & King, *Ill. Biol. Mon.* 11: 59. 1927.

Type locality: France.

Spots amphigenous, circular to angular, solitary or confluent, more or less vein-limited, 1-5 mm., at first brown to reddish-brown, becoming silvery or ashen-gray; border definite, raised, brown, 100-300  $\mu$ , the whole at times surrounded by a reddish to purplish discolored zone. Mycelium internal, hyaline to olivaceous or yellowish-brown, 2-5  $\mu$ , stromatic mycelium yellowish-brown to olive-brown. Conidiophores amphigenous but more abundant above than below, densely tufted, rupturing the epidermis, sides irregular, sufflexuous to flexuous, mostly simple but occasionally irregularly, alternately branched, arising from a compact to tuberculate stroma, brown, 30-155  $\times$  3-5  $\mu$ , mostly continuous, at times 1-2-septate; conidial scars indistinct, laterally displaced. Conidia cylindrical, tapering slightly, straight or curved, dilute-olivaceous, 30-85  $\times$  3-4.5  $\times$  2.5-4  $\mu$ , 1-9-septate.

On leaves of \**Sambucus* sp., \**S. nigra* L., *S. nigra* L. var. *laciniata* Mill., *S. racemosa* L., *S. canadensis* L. and \**S. mexicana* Presl.

*C. ticinensis* Cav. appears to be very closely related to this species and perhaps is only a variety of it.

Specimens examined: As *C. depazeoides* (Desm.) Sacc. Ellis & Ev. N. Am. Fungi 1749b, Ames, Ia.—Seym. & Earle, Ec. Fungi 476, Victoria Park, London, Canada. As *C. Sambuci* Stev. & King, Herb. Univ. Ill., Fungi of Costa Rica, No. 260 (type), Cartago.

**CERCOSPORA MANIHOTIS** P. Henn. Ann. Mus. Congo II. 2: 104. 1907.—Sacc. Syll. Fung. 22: 1421. 1913.—Welles, Am. Jour. Bot. 12: 196–218, *pls. 12–20*. 1925.

Type locality: Kisantu Congo, Africa.

Spots amphigenous, circular to effuse, 1–3 cm., somewhat obscurely concentrically zoned above, dark-brown becoming yellowish-brown to ashen-brown above, olive-brown with a purplish tinge below, frequently appearing olive-brown due to abundance of conidiophores and conidia; border indefinite. Mycelium internal, hyaline to olivaceous, 2–5  $\mu$ , stromatic mycelium yellowish-brown to olive-brown. Conidiophores amphigenous but mostly hypophyllous, solitary to fairly compactly tufted, tufts effused over the spots, rupturing the epidermis or emerging through the stomata, straight, or somewhat geniculate above, arising from a fairly compact to tuberculate stroma, dilute-olivaceous, 25–80–110  $\times$  3–5  $\mu$ , the longer ones frequently enlarged towards tips, up to 6.5  $\mu$ , continuous or 1–5-septate, simple or rarely with alternate branches arising near the bases; conidial scars distinct, minute, laterally displaced or somewhat shouldered. Conidia at first clavate, becoming cylindrical and tapering slightly, often curved, dilute-olivaceous, 25–70–90  $\times$  4.5–6.5  $\times$  4–6  $\mu$ , 2–9-septate.

On leaves of *\*Manihot utilissima* Pohl.

This species differs from *C. Henningsii* Allesch. in being amphigenous, mostly hypophyllous, and in having longer, septate, somewhat geniculate conidiophores and somewhat larger and more septate conidia. It differs from *C. Cassavae* Ellis & Ev. in producing much larger indefinitely bordered spots, in the effused tufts, and having cylindrical conidia. The conidia of *C. Cassavae* are mostly abruptly obclavate. The distinction between this species and *C. Cearae* Petch is not clear. The description of the latter species is too meager to warrant a definite comparison.

Specimens examined: Herb. Univ. Ill., Fungi of Panama No. 1085, Gamboa; No. 1113, Gamboa; No. 1230, Juan Diaz; Fungi of Costa Rica No. 496, Guapiles.

CERCOSPORA PURPUREA Cooke, Grevillea 7: 34. 1878.—Ellis & Ev. Jour. Myc. 1: 34. 1885.—Sacc. Syll. Fung. 4: 464. 1886.

Syn. *Cercospora Perseae* Ellis & Martin, Am. Nat. 18: 189. 1884.

Type locality: Georgia, Rav. Fungi Am. 190.

Spots amphigenous, circular to irregular, more or less vein-limited, 0.5–2–6 mm., dark reddish-, purplish- to blackish-brown above, similar below but usually lighter or merely brown; border same as spot, surrounding leaf tissues yellowish to brown discolored. Mycelium internal and external: external mycelium on lower leaf surface, olivaceous, 1.5–3  $\mu$ ; internal mycelium olivaceous to olive-brown, 1.5–5  $\mu$ . Conidiophores epiphyllous, densely tufted, rupturing the epidermis or effused on the external mycelium, subflexuous, arising from a tuberculate stroma, olive-brown, 20–95  $\times$  3–4  $\mu$ , continuous or 1-septate, simple or very rarely branched; conidial scars indistinct, laterally displaced and somewhat denticulate or slightly shouldered. Conidia cylindrical, tapering slightly, dilute greenish-yellow, 20–100  $\times$  2.5–3.5  $\times$  2–3.5  $\mu$ , 2–9-septate.

On leaves of *Persea palustris* (Raf.) Sarg. and \**P. gratissima* Gaertn.

The above description agrees well with the description recorded for the synonym *C. Perseae* Ellis & Mart. However, the description of *C. purpurea* Cooke is somewhat different, the conidiophores being recorded as 50–70  $\times$  4–6  $\mu$  and the conidia 40–100  $\times$  6–8  $\mu$ . The main difference in the descriptions lies in the diameters of the conidia and conidiophores. These differences are perhaps not of any consequence.

Specimens examined: Herb. Univ. Ill., Fungi of Panama No. 1191, Frijoles.

CERCOSPORA HURAE Stev. Trans. Ill. Acad. Sci. 10: 210. 1917.

Type locality: Mayaguez, Porto Rico, Herb. Univ. Ill., Porto Rican Fungi No. 478.

Spots amphigenous, circular to angular, more or less vein-limited, 0.5–2  $\times$  0.5–1 mm., brown, centers becoming grayish-

brown to gray above, numerous, concentric, raised lines marking the dead leaf tissues; border somewhat indefinite or definite, at times raised, brown to purplish. Mycelium internal, subhyaline to olivaceous,  $2-5 \mu$ , stromatic mycelium yellowish-brown. Conidiophores amphigenous but more abundant below, moderately to densely tufted, emerging through the stomata or rupturing the epidermis, simple, subflexuous to flexuous, more or less geniculate, arising from a compact to tuberculate stroma, olivaceous,  $15-95 \times 3-4.5 \mu$ , 1-2-3-septate; conidial scars minute, indistinct, laterally displaced or somewhat shouldered. Conidia cylindrical to fusiform to abruptly obclavate, straight or curved, dilute-olivaceous,  $24-87 \times 3-4.5 \times 2.5-4 \mu$ , 3-10-septate.

On leaves of *\*Hura crepitans* L.

Specimens examined: Herb. Univ. Ill., Porto Rican Fungi No. 478 (type); No. 70, Mayaguez; No. 3594, Añasco.

CERCOSPORA BRADBURYAE Young, Mycologia 8: 46. 1916.

Type locality: Rosario, Porto Rico, Herb. Univ. Ill., ex Herb. F. L. Stevens No. 446.

Spots angular to irregular, at times somewhat circular, vein-limited, confluent, 1-2-6 mm., frequently involving large areas of the leaf, brown, yellowish-brown to tan, some of the vein-limited sectors frequently being quite dark; border indefinite. Mycelium internal and external: external mycelium olivaceous, emerging with the conidiophores or through the stomata,  $1.5-3 \mu$ ; internal mycelium hyaline to olivaceous, stroma olivaceous,  $2-4.5 \mu$ . Conidiophores amphigenous, moderately to densely tufted, the denser tufts forming circular to elongate patches, rupturing the epidermis, emerging through the stomata, or effused on the external mycelium, simple or occasionally with alternate branches, walls smooth or irregular, arising from a tuberculate stroma, subhyaline to dilute-olivaceous,  $11-55 \times 3.2-4.5 \mu$ , 1-2-septate; conidial scars minute, mostly indistinct, laterally displaced or slightly shouldered. Conidia cylindrical, or tapering slightly, straight or curved, acute at both ends, subhyaline to dilute-olivaceous,  $25-115 \times 1.6-3.2 \times 1.6-2.5 \mu$ , 3-13-septate.

On leaves of *\*Bradburya pubescens* (Benth.) Kuntze.

Specimens examined: Herb. Univ. Ill., ex Herb. F. L. Stevens No. 446 (type), Rosario; No. 6558, Dos Bocas below Utuado; Nos. 3930, 6296, 479, Mayaguez; No. 263a, Santurée; No. 225a,

Hormigueras; No. 6482, Cabo Rojo; Nos. 5785, 5796, 5833, San German; No. 446a, Jayuya; No. 5609, Luguillo Forest; No. 5412, Guayama, all of Porto Rico; Fungi of Trinidad No. 826, St. Augustine.

*CERCOSPORA COSTI* Stevens, Ill. Biol. Mon. 11: 57. 1927.

Type locality: Gatun, Panama, Herb. Univ. Ill., Fungi of Panama No. 1343.

Spots amphigenous, circular, angular to irregular, blanched, or ashen due to conidiophores, large, sometimes involving a whole leaf; border definite, raised, brown to tan. Mycelium internal and external: external mycelium subhyaline to straw-colored,  $1.5\text{--}3 \mu$ ; internal mycelium subhyaline, yellowish or yellowish-

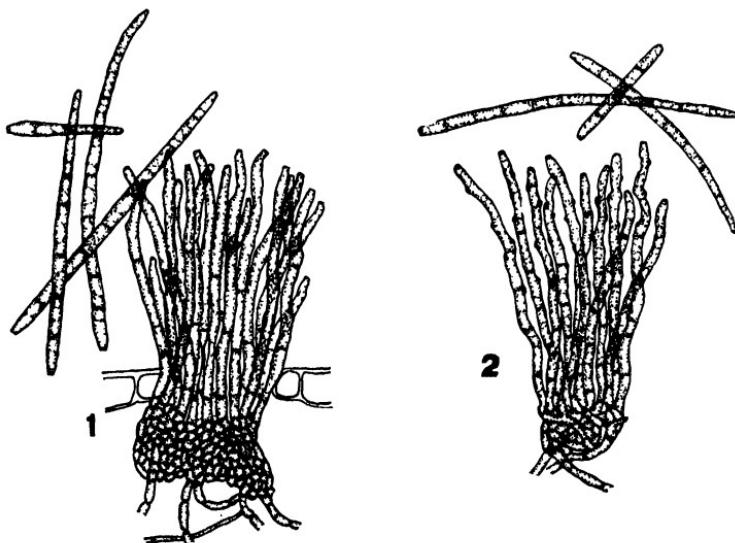


FIG. 1. *Cercospora guianensis*. Conidial scars indistinct, conidiophores simple, stroma tuberculate, conidia cylindrical.

FIG. 2. *Cercospora cylindrosporu*. Conidial scars minute, more or less indistinct, conidiophores simple, loosely stromatic, conidia cylindrical.

brown,  $2.5\text{--}5 \mu$ , stroma brown. Conidiophores amphigenous, densely tufted, emerging through the stomata and rupturing the surrounding epidermis, simple, straight, wavy margined, arising from a large tuberculate stroma (up to  $36 \mu$  in diameter), straw-colored to yellow,  $10\text{--}24 \times 2\text{--}3 \mu$ , 1-2-septate; conidial scars minute, indistinct, laterally displaced. Conidia cylindrical, bacilliform, curved, greenish-hyaline,  $30\text{--}95 \times 1.6\text{--}2.5 \times 1.6\text{--}2 \mu$ , obscurely several septate.

On leaves of *Costus* sp.

This species is readily distinguished from *C. costina* Syd.  
Specimens examined: Herb. Univ. Ill., Fungi of Panama No. 1343 (type), Gatun.

***Cercospora guianensis* Stev. & Solh. sp. nov.**

Type locality: Rockstone, British Guiana, Herb. Univ. Ill., Fungi of British Guiana No. 253.

Spots amphigenous, subcircular to irregular, 2–8 mm., light rusty-brown above, light-brown below; border indefinite. Mycelium internal, hyaline to olive-brown, 1–3.5  $\mu$ . Conidiophores amphigenous, densely tufted, emerging through the stomata or rupturing the epidermis, simple, straight, arising from a compact to tuberculate stroma, olivaceous, 20–90  $\times$  3–4  $\mu$ , 1–3-septate; conidial scars indistinct, laterally displaced. Conidia cylindrical to fusiform, or tapering slightly, straight or curved, dilute-olivaceous, 40–150  $\times$  2.5–4  $\times$  2–3.2  $\mu$ , obscurely 3–8-septate. [FIG. 1.]

On leaves of *\*Lantana* sp.

Specimens examined: Herb. Univ. Ill., Fungi of British Guiana No. 253 (type), Rockstone.

#### SECTION IV

Conidial scars minute or indistinct, conidiophores simple, stroma composed of loosely to fairly compactly interwoven hyphae or rarely none produced, conidia acicular-obclavate.

**CERCOSPORA BIXAE** Allesch. & Noack, Bol. Inst. Agron. Estoda de São Paulo 9: 2: 85. 1898.—Sacc. Syll. Fung. 16: 1066. 1902.

Type locality: Campinas, Brazil.

Spots amphigenous, circular to angular, somewhat vein-limited, 1–7 mm., light-brown or tan, to grayish-brown above, light-brown below; border definite, not raised, reddish-brown to purple, 250–750  $\mu$ . Mycelium internal and external: external mycelium subhyaline to brownish, fine, 1.5–2.5  $\mu$ ; internal mycelium subhyaline to brownish, 2–4  $\mu$ . Conidiophores amphigenous, emerging through the stomata, rupturing the epidermis or effused on the external mycelium, almost straight or flexuous, simple or occasionally with alternate branches, arising from a compact stroma, subhyaline to olivaceous, 15–52  $\times$  2.5–3  $\mu$ , continuous or 1–3-septate; conidial scars minute, rather

indistinct, somewhat warty. Conidia acicular-obclavate, dilute-olivaceous to straw-colored,  $25-130 \times 3-4 \times 1.5-2 \mu$ , 3-10-septate.

On leaves of *\*Bixa orellana* L.

Specimens examined: Herb. Univ. Ill., Porto Rican Fungi No. 56, Mayaguez; No. 3795, Rosario; No. 4845, Lares.

## SECTION VI

Conidial scars minute or indistinct, conidiophores simple, stroma composed of loosely to fairly compactly interwoven hyphae or rarely none produced, conidia cylindrical.

**Cercospora cylindrospora** Stev. & Solh. sp. nov.

Type locality: Cabo Rojo, Porto Rico, Herb. Univ. Ill., No. 6482a.

Spots amphigenous, circular to angular, more or less vein-limited and confluent, 0.5-2 mm., brown to grayish-brown; border definite, raised slightly, dark brown to reddish-brown. Mycelium internal and external: external mycelium subhyaline,  $1.5-2 \mu$ ; internal mycelium, hyaline, yellowish to brownish,  $2-4.5 \mu$ . Conidiophores mostly hypophyllous, moderately to compactly tufted, emerging through the stomata or rupturing the epidermis, simple or very rarely branched, flexuous, arising from a fairly compact stroma, dilute-brownish to yellowish-brown,  $30-105 \times 2.5-3.5 \mu$ , 1-7-septate; conidial scars minute, mostly indistinct, laterally displaced or at times somewhat shouldered. Conidia cylindrical, straight or curved, hyaline,  $40-105 \times 2-3.5 \times 2-3.2 \mu$ , 3-10-septate. [FIG. 2.]

On leaves of *\*Bradburya pubescens* (Benth.) Kuntze.

This species differs from *C. Bradburyae* Young, occurring on the same host, in producing a distinctly different type of spot and longer more septate, narrower conidiophores and more nearly cylindrical conidia.

Specimens examined: Herb. Univ. Ill. No. 6482a (type), Cabo Rojo, Porto Rico.

**Cercospora trinidadensis** Stev. & Solh. sp. nov.

Type locality: St. Augustine, Trinidad, Herb. Univ. Ill., Fungi of Trinidad, No. 839.

Spots amphigenous, angular, vein-limited, more or less confluent, 1-2 mm., brown to dark-brown; border indefinite or

definite, slightly raised, 0–300  $\mu$ , blackish-brown, surrounding leaf tissue yellowish discolored. Mycelium internal and external: external mycelium subhyaline, 1.5–2.5  $\mu$ ; internal mycelium hyaline to olivaceous, 1.5–3.5  $\mu$ . Conidiophores hypophyllous, compactly tufted, rupturing the epidermis, simple, straight, stromatic, olivaceous to brown, 15–50  $\times$  3–4  $\mu$ , continuous or 1-septate; conidial scars indistinct, laterally displaced. Conidia cylindrical, tapering slightly, olivaceous, 35–85  $\times$  3.5–5.5  $\times$  3.2–5  $\mu$ , 3–10-septate. [FIG. 3.]

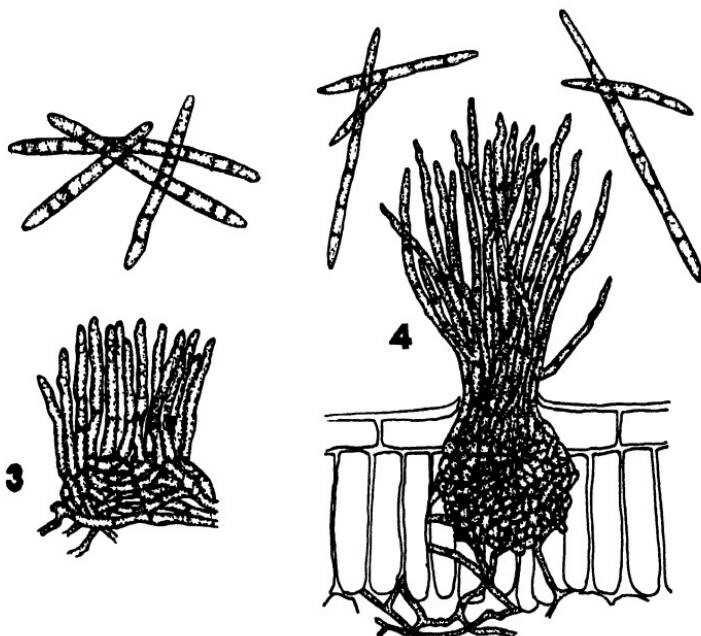


FIG. 3. *Cercospora trinidadensis*. Conidial scars indistinct, conidiophores simple, loosely stromatic, conidia cylindrical.

FIG. 4. *Cercospora Calapogonii*. Conidial scars indistinct, conidiophores alternately branched, stroma tuberculate, conidia cylindrical.

On leaves of *\*Croton gossypiifolius* Vahl.

Eight species of *Cercospora* have been recorded as occurring on various species of *Croton*. The above fungus appears to be distinct. However, it is possible that a comparison of types will put it in one of the described species.

Specimens examined: Herb. Univ. Ill. Fungi of Trinidad No. 839 (type), St. Augustine.

## SECTION X

Conidial scars minute or indistinct, conidiophores with alternate branching, stroma tuberculate, conidia cylindrical.

**CERCOSPORA ACHYRANTHIS** Sydow, Ann. Myc. 7: 171. 1909.  
—Sacc. Syll. Fung. 22: 1429. 1913.—Stevens, Trans. Ill. Acad. Sci. 10: 211. 1917.

Type locality: Ome, Musashi, Japan.

Spots amphigenous, angular, vein-limited, more or less confluent, rusty-brown to light-brown, 1–6 mm.; border indefinite. Mycelium internal, much branched, subhyaline to hyaline, very fine, 1.5–3.3  $\mu$ , stromatic mycelium brown, up to 5  $\mu$ . Conidiophores amphigenous, moderately to densely tufted, emerging through the stomata, straight to subflexuous, somewhat nodulose, arising from a fairly compact to tuberculate stroma, hyaline, subhyaline or subhyaline above and brownish toward bases, 15–130  $\times$  3–4  $\mu$ , 1–3-septate, simple or not infrequently monopodially branched, the branches well developed and usually subtending a septum; conidial scars minute, fairly distinct or indistinct, laterally displaced or irregularly shouldered. Conidia cylindrical, tapering slightly, acute at bases, subhyaline, 25–80  $\times$  3.3–5  $\times$  2.5–4.7  $\mu$ , continuous or 1–5-septate.

On leaves of *Achyranthes bidentata* Blume, *A. bidentata* Blume var. *japonica* and *\*A. crispa* Desf.

The original description records conidiophores up to 220  $\times$  4–6  $\mu$  and conidia to 125  $\times$  3–6  $\mu$  and 5–10-septate. The shape and character of the spot also differs somewhat.

Specimens examined: Herb. Univ. of Ill., Porto Rican Fungi No. 333, Guanica and No. 459a, Hormigueras.

**CERCOSPORA HIBISCI** Tracy & Earle, Bull. Torrey Club 22: 179. 1895.—Sacc. Syll. Fung. 14: 1099. 1899.

Type locality: New Orleans, La.

Spots indefinite, the conidiophores forming sooty to olive-brown, more or less vein-limited patches on the lower surface of the leaf, the whole leaf surface frequently being involved, the leaf tissue eventually turning yellow to brown. Mycelium internal and external: external mycelium subhyaline, 1–2  $\mu$ ; internal mycelium hyaline to olive-brown, 0.5–5  $\mu$ , stromatic mycelium blackish-brown. Conidiophores amphigenous but more abundant on the lower surface, compactly tufted, emerging

through the stomata, straight to subflexuous, more or less irregularly constricted, arising from a compact to tuberculate stroma, dilute-fuliginous,  $25-140 \times 3-5 \mu$ , 1-6-septate, more or less branched, the branches alternate; conidial scars minute, indistinct, laterally displaced. Conidia cylindrical to obclavate, straight or curved, dilute-fuliginous,  $25-90 \times 3.5-5.5 \times 3-5 \mu$ , 1-7-septate, more or less constricted at septa.

On leaves of *\*Hibiscus esculentus* L.

This species was reported by Miss Young, *Mycologia* 8: 44. 1916, as occurring on *Hibiscus tiliaceus* L. Her specimens were examined and all proved to be *C. hibiscina* Ellis & Ev. This species is quite distinct from the one described above.

Specimens examined: Herb. Univ. Ill., Fungi of British Guiana No. 175, Tumatumari; Porto Rican Fungi No. 5030, Quebradillas; No. 5229, Aguadilla; No. 6465, Cabo Rojo.—Seym. & Earle, Ec. Fungi No. 462, Ocean Springs, Miss.

**Cercospora Calopogonii** Stev. & Solh. sp. nov.

Type locality: St. Augustine, Trinidad, Herb. Univ. Ill., Fungi of Trinidad No. 836.

Spots amphigenous, subcircular to irregular, 3-7 mm., brown to dirty-brown; border indefinite or definite, darker than spot. Mycelium internal and external: external mycelium, subhyaline to olivaceous, emerging with the conidiophores above, through the stomata below,  $1.5-3 \mu$ ; internal mycelium subhyaline, brown in stromata,  $1.5-3.5 \mu$ . Conidiophores mostly epiphyllous and then fairly densely tufted and coremioid, when below solitary or loosely tufted, lax and creeping, emerging through the stomata or rupturing the epidermis, straight to flexuous, arising from a compact to tuberculate stroma, olivaceous,  $50-150 \times 2.2-3.5 \mu$ , 2-8-septate, more or less branched, the branches alternate and well developed; conidial scars minute, indistinct, laterally displaced or slightly shouldered. Conidia cylindrical to fusiform, at times somewhat curved, subhyaline to olivaceous,  $25-90 \times 2.5-3.5 \times 2-3.5 \mu$ , 2-7-septate. [FIG. 4.]

On leaves of *\*Calopogonium* sp.

Specimens examined: Herb. Univ. Ill., Fungi of Trinidad No. 836 (type), St. Augustine.

**CERCOSPORA PANCRATII** Ellis & Ev. *Jour. Myc.* 3: 15. 1887.—*Sacc. Syll. Fung.* 10: 654. 1892.—Tracy & Earle, *Miss. Agric. Exp. Sta. Bull.* 34: 120. 1895.

Syn. *Cercospora Hymenocallidis* Pat. Bull. Soc. Myc. Fr. 28: 142. 1912.

Type locality: Louisiana, Langlois No. 656.

Spots amphigenous, circular to elliptical, more or less confluent, 2–14 mm., brown, blackish-brown, reddish-brown or red; border definite, raised, brown to blackish, fairly wide, or indefinite. Mycelium internal and external: external mycelium subhyaline, 1.5–3  $\mu$ , emerging with the conidiophores; internal mycelium subhyaline to olive-brown to brown, 1.6–4.5  $\mu$ , stroma black in mass. Conidiophores amphigenous, densely tufted, emerging through the stomata, or at times arising from the external mycelium, straight to flexuous, arising from a large tuberculate stroma, subhyaline to dilute-olivaceous becoming brownish, 25–110  $\times$  2.5–3.5–4.5  $\mu$ , 1–3-septate, branched, the branches alternate, somewhat difficult to observe; conidial scars more or less indistinct, laterally displaced or somewhat shouldered. Conidia cylindrical-bacilliform, tapering slightly, subhyaline to greenish, 40–130  $\times$  2–3.5  $\times$  1.5–2.5  $\mu$ , 3–9-septate.

On leaves of \**Hymenocallis* sp., *H. crassifolia* Herb., *H. littoralis* Salisb. and *Crinum americanum* L.

*C. Hymenocallidis* Pat. has not been seen but the description indicates that it is not distinct from *C. Pancratii* Ellis & Ev. It is, therefore, recorded as a synonym.

The specimens listed below were reported by Miss Young, Mycologia 8: 44. 1916, as *C. Amaryllidis* Ellis & Ev.

Specimens examined: Herb. Univ. Ill., ex Herb. F. L. Stevens No. 244, Santuree, Porto Rico and No. 836, Caoma, Porto Rico.

CERCOSPORA BIFORMIS Peck, Bull. Torrey Club. 36: 156. 1906.—Sacc. Syll. Fung. 22: 1414. 1913.—Stev. Trans. Ill. Acad. Sci. 10: 210. 1917.

Type locality: Batesville, Arkansas.

Spots amphigenous, irregular to angular, vein-limited, sometimes confluent, 3–5 mm., olivaceous, becoming dirty-brown; border indefinite. Mycelium internal and external: external mycelium dilute-brownish, 1.5–2.5  $\mu$ ; internal mycelium irregular, subhyaline to dilute-olivaceous, 1.5–5  $\mu$ , stromatic mycelium dark reddish-brown as seen in mass. Conidiophores amphigenous, moderately to densely tufted, emerging through the stomata, rupturing the epidermis or effused on the external mycelium, flexuous, arising from a tuberculate stroma, olive-

brown to reddish-brown,  $20-70 \times 3-5 \mu$ , continuous or 1-3-septate, more or less branched, the branches short and irregularly alternate; conidial scars minute, indistinct. Conidia cylindrical, subhyaline to dilute-brownish,  $30-100 \times 2.5-3.5 \times 2.5-3 \mu$ , 3-10-septate.

On leaves of *Passiflora incarnata* L. and \**P. sexflora* A. Juss.

The specimen examined does not agree very well with the original description of the species. Peck records two types of conidia. Only cylindrical conidia were observed in the Porto Rican specimen. Three other species of *Cercospora* are recorded on *Passiflora* spp. None of the descriptions of these agree well with the fungus examined. From a comparison of the descriptions it is highly improbable that they are all distinct. Until further studies of these can be made the Porto Rican specimen is left as originally determined.

Specimens examined: Herb. Univ. Ill., Porto Rican Fungi No. 1140, Mayaguez.

## SECTION XII

Conidial scars minute or indistinct, conidiophores with alternate branching, stroma composed of loosely to fairly compactly interwoven hyphae or rarely none produced, conidia abruptly obclavate.

CERCOSPORA CASSAVAE Ellis & Ev. Bull. Torrey Club 22: 438. 1895.—Sacc. Syll. Fung. 14: 1104. 1899.—Young, Mycologia 8: 44. 1916.

Type locality: Florida, G. V. Nash, Summer of 1895, No. 1950.

Spots amphigenous, circular to angular, somewhat vein-limited, 2-8 mm., olive-brown to rusty-brown above, below at times with a bluish tinge due to bloom on leaf; border definite, raised, olive-brown to rusty-brown,  $75-250 \mu$ , a narrow strip of the surrounding tissue at times yellowish-green. Mycelium internal, regular to irregular, hyaline to olive-brown,  $1.5-7.5 \mu$ , stromatic mycelium yellowish-brown, to olive-brown, dark reddish-brown in mass. Conidiophores amphigenous, somewhat aggregated along the veins, solitary to fairly densely tufted below, densely tufted above, emerging through the stomata below, rupturing the epidermis above, straight, arising from a loose to compact almost tuberculate stroma, subhyaline, yellowish to olivaceous,  $25-80 \times 3-5 \mu$ , continuous or 1-3-septate, mostly simple, but

not infrequently with branches arising at or near the bases; conidial scars minute, laterally displaced and somewhat dentate or at times somewhat shouldered, more or less indistinct. Conidia abruptly obclavate to cylindrical, dilute-yellowish to olivaceous,  $20-65 \times 4-6 \times 3-5 \mu$ , continuous or 1-6-septate.

On leaves of *\*Manihot* sp. and *\*M. utilissima* Pohl.

Three other species of *Cercospora* are recorded as growing on *Manihot*. These are *C. Henningsii* Allesch., *C. Manihotis* P. Henn. and *C. Cearae* Petch. The descriptions of these are too meager to judge whether or not they are distinct. From what description there is, it appears quite doubtful that they are so.

Specimens examined: Herb. Univ. Ill., Fungi of Costa Rica No. 821, Port Limon; Porto Rican Fungi No. 254a, Santuree; Fungi of Panama No. 1100, Pedro Miguel.

### SECTION XIII

Conidial scars minute or indistinct, conidiophores with alternate branching, stoma composed of loosely to fairly compactly interwoven hyphae or rarely none produced, conidia cylindrical.

**CERCOSPORA HIBISCINA** Ellis & Ev. Proc. Acad. Sci. Phila. 1895: 438.—Sacc. Syll. Fung. 14: 1099. 1899.

Type locality: Mexico.

Spots indefinite, the conidiophores forming sooty, velvety patches on the lower surface of the leaf and frequently covering the major portion of the leaf, the leaf tissue eventually becoming brown. Mycelium internal and external: external mycelium olivaceous,  $2-3 \mu$ ; internal mycelium subhyaline,  $1.5-3 \mu$ . Conidiophores hypophylloous, loosely tufted, emerging through the stomata or effused on the external mycelium, straight, lax, somewhat geniculate towards tips and at times also for short distances at various points on the conidiophore, non-stromatic or loosely stromatic, dark-brown,  $240-600 \mu$  to 1 mm.  $\times 3-4 \mu$ , 9-25-35-septate, branched, the branches alternate and well developed; conidial scars minute, laterally displaced or somewhat shouldered. Conidia cylindrical or tapering slightly, subhyaline, olivaceous to reddish-brown,  $20-70 \times 3-4.5 \times 3-4 \mu$ , 2-6-mostly 3-septate.

On leaves of *\*Hibiscus tiliaceus* L.

Specimens examined: Herb. Univ. Ill., Porto Rican Fungi No. 8073, Dos Bocas; No. 310, Las Marias; No. 8456, Aibonito;

No. 8501, Sigante; No. 8962, Maricao; No. 9484 (locality not given); No. 9147, Penuelas; Herb. Univ. Ill. ex Herb. F. L. Stevens No. 3630, Rio Maricao above Maricao; No. 3793, Rosario; No. 4797, Maricao; No. 6564, Dos Bocas; No. 6618, Dos Bocas, all of Porto Rico.

CERCOSPORA PIPTURI Stev. & Glick, Bull. Bernice P. Bishop Mus. 19: 155. 1925. *Fig. 33, b.*

Type locality: Kauai; Kalalau trail, Hawaii, Herb. Univ. Ill., Hawaiian Fungi No. 538.

Spots indefinite, the conidiophores forming brown to sooty, velvety patches on the lower surface of the leaf and occasionally on the upper surface. Mycelium internal and external: external mycelium hyaline to brown, of two kinds, fine and regular or coarser and beaded, the latter type of mycelium occurring especially on the upper leaf surface, 1.5–5  $\mu$ ; internal mycelium subhyaline, 1.5–3  $\mu$ . Conidiophores hypophyllous or occasionally also on the upper leaf surface (not necessarily above the lower spots), loosely tufted, emerging through the stomata or arising from the external mycelium, lax, intertwined with each other and with trichomes, non-stromatic, fuscus, at times with a reddish tinge, 100–550  $\times$  3–6  $\mu$ , 10–25-septate, branched, the branches alternate, well developed; conidial scars mostly indistinct, laterally displaced or at times slightly shouldered. Conidia cylindrical, fusiform to obclavate, often curved, about the same color as the conidiophores or somewhat lighter to olivaceous, 40–115  $\times$  4.5–8  $\times$  4.5–6  $\mu$ , the greatest diameter usually obtaining near or just below the middle, 2–8-septate.

On leaves of *\*Pipturus gaudichaudianus* Wedd. (*P. albidus* A. Gray).

Specimens examined: Herb. Univ. Ill., Hawaiian Fungi No. 538 (type), Kalalau trail, Kauai; No. 766, between Hilo and Kilauea, Hawaii; No. 894, Kapapalo ranch, Hawaii; No. 1020, between Kona and Waimea, Hawaii; No. 713, Olympus, Oahu.

CERCOSPORA RIGOSPORA Atkinson, Jour. Elisha Mitch. Soc., 8: 65. 1891.—Sacc. Syll. Fung. 10: 635. 1892.—Underw. & Earle, Ala. Agric. Exp. Sta. Bull. 80: 150. 1897.

Type locality: Auburn, Alabama.

Spots indefinite, the conidiophores forming olivaceous patches on the lower surface of the leaf and to a lesser extent on the

upper leaf surface, the infected leaf tissue eventually becoming yellowish to brown. Mycelium internal and external: external mycelium subhyaline, 1.5–3  $\mu$ ; internal mycelium subhyaline to olive-yellow, 1.5–4.5  $\mu$ . Conidiophores amphigenous but mostly hypophyllous, moderately tufted, emerging through the stomata, flexuous, more or less torulose, non-stromatic, or arising from a small stroma, olivaceous, 20–110  $\times$  3–5  $\mu$ , 1–7-septate, branched, the branches alternate, short or well developed; conidial scars minute, indistinct, laterally displaced or somewhat shouldered. Conidia cylindrical or tapering slightly, dilute-olivaceous, 15–82  $\times$  2.5–3.5–(4.5)  $\times$  2–3  $\mu$ , 1–10-septate.

On leaves of *\*Solanum nigrum* L.

Specimens examined: Herb. Univ. Ill. No. 5316, Rio Piedras, Porto Rico; Fungi of Costa Rica No. 6, San Jose; No. 450, Peralta.

CERCOSPORA CRUENTA Sacc. *Michelia* 2: 149. 1880; *Fungi Ital.* Pl. 686; *Fungi Carol.* Pl 2, fig. 8: *Syll. Fung.* 4: 435. 1886.—Ellis & Ev. *Jour. Myc.* 2: 1. 1886.—Atkinson, *Jour. Elisha Mitch. Soc.* 8: 56. 1891.—Jackson, *Del. Agric. Sta. Bull.* 83: 22–24. 1908. *Figs. 11–12.*—Heald & Wolf, *Bu. Pl. Ind. Bull.* 226: 49. 1912. *Pl. 1, fig. 2.*—Schwarze, *N. J. Agric. Sta. Bull.* 313: 134, *fig. 801.* 1917.

Type locality: South Carolina.

Spots definite or indefinite, circular, subcircular, to irregular, more or less vein-limited and confluent, 1–10 mm., pale-green, yellowish, olive-brown, rusty-brown, to reddish-brown, appearing olivaceous below due to the conidiophores; border indefinite. Mycelium internal and at times external; external mycelium subhyaline, 1.5–2.5  $\mu$ ; internal mycelium subhyaline to olivaceous, 2–5  $\mu$ . Conidiophores amphigenous, moderately to fairly compactly tufted, tufts scattered over almost entire leaf surface, emerging through the stomata or effused on the external mycelium, straight to subflexuous, non-stromatic or arising from a loose stroma, olivaceous, 20–65  $\times$  3–4.5  $\mu$ , continuous or 1–3-septate, more or less branched, the branches alternate; conidial scars mostly minute, indistinct, laterally displaced and somewhat denticulate. Conidia cylindrical or tapering slightly, 35–140  $\times$  3–4.5  $\times$  2.5–4  $\mu$ , 1–13-septate.

On leaves, stems and pods of *\*Phaseolus* sp., *\*P. vulgaris*, L., *P. lunatus* L., *P. mungo* L., *Dolichos* sp. and *\*Vigna catjang* Walp.

The fungus which Welles has reported from the Philippine

Islands as *C. cruenta*, *Phytopathology* 14: 351-358, figs. 1-2 and *Am. Jour. Bot.* 12: 208-218, does not appear to belong here. His drawings do not in the least resemble the species.

*C. Dolichi* Ellis & Ev. is perhaps identical with this species.

Specimens examined: Ellis & Ev. *N. Am. Fungi* 2294 (locality not given).—Seym. & Earle, *Ec. Fungi* 413, Ocean Springs, Miss.; 514, Auburn, Ala.—Herb. Univ. Ill., *Fungi of British Guiana* No. 18, Georgetown.

## SECTION XVII

Conidial scars minute or indistinct, conidiophores with alternate and opposite branching, stroma composed of loosely to fairly compactly interwoven hyphae or rarely none produced, conidia acicular-obclavate.

*CERCOSPORA BORRERIAE* Ellis & Ev. *Proc. Acad. Sci. Phila.* 1894: 379. 1894.—Tracy & Earle, *Miss. Bull.* 35: 116. 1895.—*Sacc. Syll. Fung.* 11: 627. 1895.

Type locality: Biloxi, Miss.

Spots? Mycelium internal, very irregular, olive-brown, appearing black in the stromata in mass,  $2-7 \mu$ . Conidiophores amphigenous, emerging through the stomata or rupturing the epidermis, straight or subflexuous, with more or less bulbous bases, arising from a loose to compact to almost tuberculate stroma, reddish-brown,  $35-140 \times 3-5 \mu$ , continuous or 1-6-septate, more or less branched, the branches alternate and opposite, well developed; conidial scars laterally displaced and somewhat warty or shouldered becoming more or less indistinct. Conidia acicular-obclavate, subhyaline,  $35-160 \times 3-4 \times 1-1.5 \mu$ , somewhat obscurely 3-15-septate.

On leaves of *\*Borreria* sp. and *Spermacoce ocymoides* Burm (*Borreria micrantha*).

The material examined was such that it was impossible to determine the nature of the spot or whether an external mycelium was present.

Specimens examined: Herb. Univ. Ill., *Fungi of Trinidad* No. 858, Port of Spain.

## SECTION XIX

Conidial scars minute or indistinct, conidiophous with alternate and opposite branching, stroma composed of loosely to fairly compactly interwoven hyphae or rarely none produced, conidia cylindrical.

**Cercospora Cayaponiae** Stev. & Solh. sp. nov.

Type locality: Rosario, Porto Rico, Herb. Univ. Ill., Porto Rican Fungi No. 3777.

Spots indefinite, the conidiophores forming sooty to reddish-brown, vein-limited patches on the lower surface of the leaf, the infected leaf tissue eventually becoming yellowish to brownish discolored. Mycelium internal and external: external mycelium abundant, emerging through the stomata, hyaline to brown,  $1.5-3.5 \mu$ ; internal mycelium subhyaline, fine,  $1-2.5 \mu$ . Conidiophores amphigenous, mostly hypophyllous, moderately to fairly densely tufted, emerging through the stomata, flexuous, more or less geniculate, non-stromatic, reddish-brown,  $30-175 \times 3.5-5.5 \mu$ , 1-5-septate towards bases, much branched, the branches both alternate and opposite but mostly alternate; conidial scars minute but distinct, laterally displaced or shouldered. Conidia cylindrical, dilute-yellowish to yellowish-brown,  $25-110 \times 4-5 \times 3.5-5 \mu$ , 1-6-septate. [FIG. 9.]

On leaves of *\*Cayaponia* ssp.

The conidia of this fungus at times produce secondary conidia near the tips.

Specimens examined: Herb. Univ. Ill., Porto Rican Fungi No. 3777 (type), Rosario; No. 4815, Maricao. (These specimens were previously reported as *C. cucurbiticola* P. Henn.)

## SECTION XX

Conidial scars distinct, over  $2 \mu$  in diameter, conidiophores simple, stroma tuberculate, conidia acicular-obclavate.

**CERCOSPORA NICOTIANAE** Ellis & Ev. Proc. Acad. Sci. Phila. 1893: 170. 1893.—Sacc. Syll. Fung. 11: 628. 1895.—Sturgis, Ann. Rep. Conn. Agric. Exp. Sta. 20: 273-278, pls. 7, 8, figs. 1-3. 1897.—Eng. Bot. Jahr. 28: 279. 1901.—Hedwigia 41: 310. 1903.—Heald & Wolf, Bü. Pl. Ind. Bull. 226: 105. 1912.

—Bull. Soc. Path. Veg. Fr. 5: 45. 1918. *Fig. 1, a-e.*—Welles, Am. Jour. Bot., 12: 202. 1925.

Type locality: Raleigh, North Carolina.

Spots, amphigenous, circular to angular to irregular, more or less vein-limited and confluent, 0.5–5 mm., dark centered, the spots frequently appearing olivaceous to sooty due to the abundance of conidiophores; border definite, raised, blackish-brown to brown above, light-brown below. Mycelium internal and external: external mycelium arising from the internal and emerging with the conidiophores or arising directly from the conidiophores, subhyaline, 1.5–3  $\mu$ ; internal mycelium hyaline to yellowish-brown, 1–6.5  $\mu$ . Conidiophores amphigenous, moderately to compactly tufted, rupturing the epidermis, straight to flexuous, geniculate, simple, arising from a fairly compact to tuberculate stroma, brown with reddish tinge, 20–285–(615)  $\times$  3.5–5  $\mu$ , continuous or 1–10–(20)-septate; conidial scars distinct, shouldered or laterally displaced. Conidia narrowly acicular, acute at tip, hyaline, 35–245  $\times$  3–5  $\times$  1.5–2  $\mu$  (secondary conidia 2.5–3.5  $\mu$  at base), 5–26-septate.

On leaves of *\*Nicotiana Tabacum* L., *N. ssp.* and *N. repanda* Willd.

The specimen collected by J. A. Stevenson at Bayaman, Porto Rico, Herb. Univ. Ill. No. 5517 has for the most part very long straight, non-geniculate, many-septate conidiophores. The maximum numerical expressions of length and septation is recorded in the parentheses above. The ordinary conidiophores of the species were also found, the two types emerging together. It seems probable, therefore, that both types of conidiophores are common to the species. It is possible, however, that two different fungi are being dealt with.

Specimens examined: Herb. Univ. Ill., Porto Rican Fungi No. 7270, Quebradillas; No. 7980, Dos Bocas; No. 7612, Ste. Ana; No. 5517, J. A. Stevenson, Bayamon, Porto Rico; Fungi Malayana No. 123, Mt. Maquiling, near Los Baños, Province Laguna, Philippine Islands.

#### SECTION EUCERCOSPORA

Conidial scars distinct, over 2  $\mu$  in diameter, conidiophores simple, stroma composed of loosely to fairly compactly interwoven hyphae or rarely none produced, conidia acicular-obclavate.

***Cercospora Atkinsonii* Stev. & Solh. sp. nov.**

Syn. *Cercospora althaeina* Sacc. var. *Modiolae* Atkinson, Jour. Elisha Mitch. Soc. 8: 60. 1891.—Underw. & Earle, Ala. Bull. 80: 141. 1897.

Type locality: Alabama.

Spots amphigenous, circular to angular, vein-limited, 1–3 mm., olivaceous, becoming brown to grayish centered; border more or less indefinite, of a darker-brown than the spot proper. Mycelium internal, irregular, subhyaline, 2–6.5  $\mu$ , stromatic my-

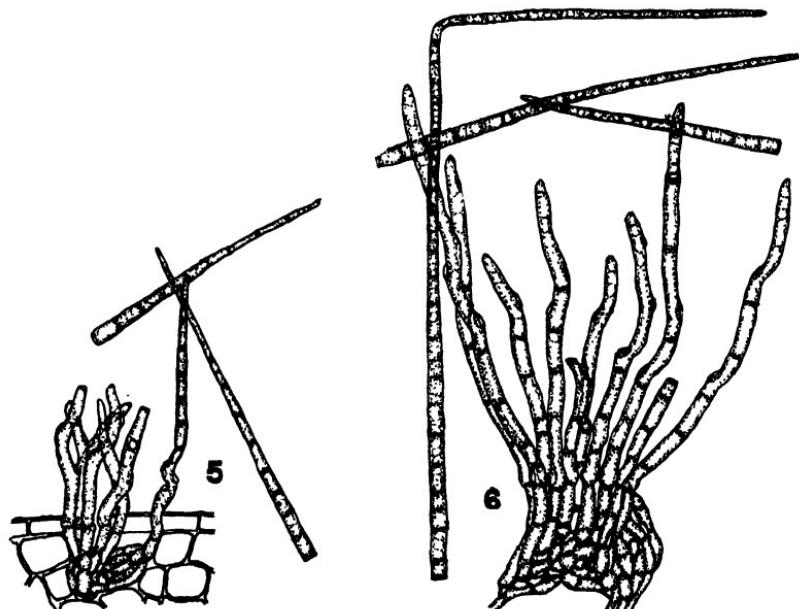


FIG. 5. *Cercospora Atkinsonii*. Conidial scars over 2  $\mu$ , conidiophores simple, stroma loose to compact, conidia acicular.

FIG. 6. *Cercospora malayensis*. Conidial scars over 2  $\mu$ , conidiophores simple, stroma loose to compact, conidia acicular.

celium subhyaline to brownish. Conidiophores amphigenous, loosely to moderately tufted, emerging through the stomata or rupturing the epidermis, simple, straight, more or less geniculate, arising from a fairly compact stroma, subhyaline to brownish, 15–52  $\times$  4.5–5  $\mu$ , continuous; conidial scars fairly distinct, shouldered. Conidia acicular, subhyaline, 50–105  $\times$  3–3.5  $\times$  1.5–2  $\mu$ , 5–11-septate. [FIG. 5.]

On leaves of *\*Modiola multifida* Moench.

The type of the variety has not been seen but the material at

hand agrees very well with the description of it. It appears to be quite distinct from *C. althaeina* Sacc. having shorter conidiophores, acicular conidia and much narrower conidia. It is also distinct from *C. Modiolae* Tharp, which, from the description, has much thicker conidia and longer conidiophores. The new species is named after the author of the variety, the late Prof. Atkinson.

Specimens examined: Herb. Univ. Ill., Hawaiian Fungi No. 1047, Waimea and No. 1082, Hamakua, upper ditch trail. (These specimens were issued under *C. althaeina* Sacc., Bull. Bernice P. Bishop Mus., 19: 154. 1925.)

*CERCOSPORA ATRICINCTA* Heald & Wolf, Mycologia 3: 14. 1911.

Type locality: Victoria, Texas, 2506.

Spots amphigenous, subcircular to irregular, more or less vein-limited, 0.5–4 mm., at first brown, becoming dirty-white; border definite, raised, narrow, light-brown below, purplish- to blackish-brown above, frequently with a broad marginal discolored zone which is olivaceous below and purplish- to blackish-brown above. Mycelium internal and external: external mycelium hyaline to brownish, fine, 1.5–3  $\mu$ , internal mycelium hyaline to brownish, 1.5–6.5  $\mu$ , stromatic mycelium appearing reddish-brown in mass. Conidiophores amphigenous, moderately tufted, emerging through the stomata or rupturing the epidermis, straight to subgeniculate, arising from a fairly compact stroma, reddish-brown, 35–104  $\times$  3.5–4.5  $\mu$ , 1–5-septate, simple or very rarely with opposite branches; conidial scars laterally displaced and more or less warty, or less commonly somewhat shouldered, fairly distinct. Conidia acicular, subhyaline to dilute-bluish or yellowish, 40–140  $\times$  3.5–4  $\times$  1.5–2  $\mu$ , 5–15-septate.

On leaves of *\*Zinnia* sp.

The original description describes the conidia as being dilute brownish and 100–200  $\times$  4–4.5  $\mu$ .

Specimens examined: Herb. Univ. Ill. No. 3130, J. A. Stevenson, Espinosa, Porto Rico.

*CERCOSPORA CANESCENS* Ellis & Martin, Am. Nat. 16: 1003. 1882.—Ellis & Ev. Jour. Myc. 1: 21. 1885; 3: 19. 1887; 8: 73. 1902.—Wint. & Dem. Hedwigia 24: 203. 1885.—Sacc.

Syll. Fung. 4: 435. 1886.—Atkinson, Jour. Elisha Mitch. Soc. 8: 48. 1891.—Heald & Wolf, Bu. Pl. Ind. Bull. 226: 37, pl. 2, figs. 5-6; pl. 9, fig. 3. 1912.—Schwarze, N. J. Agric. Exp. Sta. Bull. 313: 132, fig. 788. 1917.

Type locality: Newfield, N. J.

Spots amphigenous, circular to angular to irregular, vein-limited, confluent, 0.5-8 mm., brown, reddish-brown, becoming grayish-brown to white centered; border definite, raised, rusty-brown to reddish-brown. Mycelium internal, hyaline, subhyaline to yellowish-brown, brown in stromata, 1.5-4  $\mu$ , up to 7  $\mu$  in stromata. Conidiophores amphigenous, moderately to fairly compactly tufted, emerging through the stomata or rupturing the epidermis, simple or rarely branched, with or without a bulbous base, subflexuous, geniculate, arising from a loose to compact stroma, brown to olivaceous, 20-175-210  $\times$  3-6.5  $\mu$ , continuous or 1-7-septate; conidial scars distinct, shouldered or somewhat laterally displaced, mostly scattered. Conidia narrowly acicular to acicular-obclavate or at times somewhat cylindrical or fusiform, hyaline to greenish, 35-210  $\times$  2.5-5  $\times$  1-2.5  $\mu$ , 3-20-septate.

On leaves of *\*Phaseolus* sp., *\*P. lunatus* L., *\*P. vulgaris* L., *\*Vigna catjang* Walp., *V. glabra* Savi, *Amaranthus* sp., *Ricinus communis* L., and on dead stems of *Lycopersicum esculentum* Mill. and *Petunia parviflora* Juss.

Conidial measurements given above do not agree well with those of the original description. However, they agree with those given by Heald and Wolf who have based their determination on a comparison of their material with the specimens in the herbarium of the New York Botanical Garden.

Specimen No. 5130, Herb. Univ. Ill., collected by J. A. Stevenson at Rio Piedras, Porto Rico, April 5, 1916, and recorded as *C. canescens* is not this species. The material was too poor to permit definite determination beyond this. This, as far as the authors know is the only record of *C. canescens* as occurring on *Dolichos Lablab* L.

Specimen No. 5835 of the same collector on *Vigna catjang* Walp., recorded as *C. canescens* is a *Helminthosporium*. The specimen was collected at Garrochales, Porto Rico, Dec. 2, 1916.

Specimens examined: As *C. cruenta* Sacc., Herb. Univ. Ill., ex Herb. F. L. Stevens No. 261, Santurée, Porto Rico.—Seym.

& Earle, Ec. Fungi No. 507, Auburn, Ala. As *C. Vignae* Racib. (?), Herb. Univ. Ill., J. A. Stevenson No. 2110, Rio Piedras, Porto Rico. As *C. canescens* Ellis and Mart., Herb. Univ. Ill., Porto Rican Fungi No. 5872, Guayanilla; No. 32964, ex Herb. U. S. Dept. Agric. D. V. P. P. No. 1199, Manhattan, Kans.—Rabenh.-Winter, Fungi Eu. No. 3788, Perryville, Mo.

**CERCOSPORA ERECHTITIS** Atkinson, Jour. Elisha Mitch. Soc. **8**: 66. 1891.—Sacc. Syll. Fung. **10**: 629. 1892.—Underw. & Earle, Ala. Agric. Exp. Sta. Bull. **80**: 145. 1897.—Stevens, Ill. Biol. Mon. **11**: 57–58. 1927.

Type locality: Auburn, Alabama.

Spots amphigenous, circular, 0.5–2 mm., brown, at times whitish centered: border definite, raised, about the same color as the spot, 100–150  $\mu$ . Mycelium internal and external: external mycelium hyaline to subhyaline, 1.5–3.5  $\mu$ ; internal mycelium subhyaline to brown, 3–8  $\mu$ . Conidiophores amphigenous, solitary or moderately tufted, emerging through the stomata, rupturing the epidermis or effused on the external mycelium, straight to flexuous, at times geniculate, non-stromatic or arising from a loose stroma, reddish-brown, 30–140  $\times$  3.5–5  $\mu$ , continuous or 1–6-septate, simple or rarely with either alternate or opposite branches; conidial scars distinct, laterally displaced and somewhat warty or shouldered. Conidia acicular, to acicular-obclavate, subhyaline, 45–140  $\times$  2.5–4  $\times$  1.5–2  $\mu$ , 6–20-septate.

On leaves of *\*Erechtites* sp. and *E. praealta* Rafin.

The original description records the conidiophores as being epiphyllous, 50–240  $\times$  4  $\mu$  and the conidia 70–230  $\times$  4  $\mu$ .

Specimens examined: Herb. Univ. Ill., Fungi of Costa Rica No. 556. Experiencia Farm.

**CERCOSPORA PLANTAGINIS** Sacc. Michelia **1**: 267. 1878; Fungi Ital. Pl. **666**; Syll. Fung. **4**: 454. 1886.—Ellis & Ev. Jour. Myc. **1**: 19. 1885.—Lindau in Rabenh. Krypt.-Fl. **1<sup>9</sup>**: 133. 1910, fig. p. 134.

Type locality: Italy.

Spots amphigenous, circular to angular to elliptical, somewhat vein-limited, 0.5–4 mm., brownish-gray to gray or whitish; border definite, raised, brown. Mycelium internal and external; external mycelium hyaline, fine 1–2  $\mu$ ; internal mycelium sub-

hyaline to brown, frequently forming mats,  $1.5\text{--}6.5\ \mu$ . Conidiophores amphigenous, loosely to moderately tufted, emerging through the stomata or at times rupturing the epidermis, simple, more or less geniculate, arising from a loose to compact stroma, brown,  $30\text{--}140 \times 3\text{--}5\ \mu$ , continuous or 1-3-septate, conidial scars distinct, laterally displaced or somewhat shouldered. Conidia acicular, subhyaline to hyaline,  $40\text{--}200 \times 2.5\text{--}3.5 \times 1.5\text{--}2\ \mu$ , 6-20-septate.

On leaves of *\*Plantago* sp., *\*P. lanceolata* L., *\*P. major* L. and *P. lusitanica* L.

Specimens examined: Herb. Univ. Ill. No. 20076, ex Herb. Mo. State Univ., Columbia, Mo., ex herb. F. L. Stevens, New Brunswick, N. J.; Hawaiian Fungi No. 433, Upper Waimea Canyon, Kauai.

CERCOSPORA BLOXAMI Berk. & Br. Ann. Mag. Nat. Hist. V. 9: 183. 1882; Cooke, Grevillea 11: 14. 1882.—Sacc. Syll. Fung. 4: 433. 1886.—Cooke, Fung. Pests, pl. 7, fig. 97.—Kirchner, Krankh. Kulturpfl. 306. 1906. Atlas 3: pl. 12, figs. 1-2.—Lindau in Rabenh. Krypt.-Fl. 9: 98. 1910.—Heald & Wolf, Bu. Pl. Ind. Bull. 226: 38. 1912.—Young, Mycologia 8: 43. 1916.—Ann. Myc. 22: 193. 1924.

Type locality: Twycross, England, Berkeley, No. 1979.

Spots amphigenous, circular to angular, more or less confluent, 1-5 mm., light-green, light-brownish to sordid-white; border definite, not raised or distinctly raised, brown. Mycelium internal and external: external mycelium rather sparse, emerging with the conidiophores, hyaline,  $2\text{--}3.2\ \mu$ ; internal mycelium hyaline, brown just below the conidiophores,  $2\text{--}6.5\ \mu$ . Conidiophores amphigenous, loosely to fairly compactly tufted, emerging through the stomata or rupturing the epidermis, or at times arising from the external mycelium, simple or rarely alternately branched, straight to flexuous, more or less geniculate, arising from a loose stroma, dilute-brown to brown,  $25\text{--}260 \times 3.2\text{--}5.5\ \mu$ , continuous or 1-8-septate; conidial scars distinct, usually rather distantly spaced, shouldered. Conidia acicular to acicular-obclavate, straight or somewhat flexuous, greenish-hyaline,  $25\text{--}200 \times 2.5\text{--}3.5 \times 1\text{--}2\ \mu$ , 3-22-septate.

On leaves of *\*Brassica* sp., *\*B. juncea* Coss., *\*B. nigra* Koch., *B. campestris* L. (*B. napus* L. and *B. Rapa* L.), *B. oleracea capitata* L.

Specimens examined: Seym. & Earle, Ec. Fungi 255, New Brunswick, N. J. (Compared with original specimen by Prof. Geo. Massee.)—Herb. Univ. Ill. No. 6782, Rio Piedras, Porto Rico, J. A. Stevenson; ex Herb. F. L. Stevens No. 449, Bayamon, Porto Rico; No. 5121, Quebradillas, Porto Rico.

CERCOSPORA ARCTI-AMBROSIAE Halsted, Bull. Torrey Club 20: 251. 1893.—Seym. & Earle, Ec. Fungi No. 296. 1893.

Syn. *Cercospora Arctii* Stevens, Bull. Bernice P. Bishop Mus., 19: 154. 1925.

Type locality: *Cercospora Arcti-ambrosiae* Halsted, New Brunswick, N. J., Sept., 1892, F. L. Stevens, Seym. & Earle, Ec. Fungi 296. *Cercospora Arctii* Stev. Hawaii: Kukuihaele, Herb. Univ. Ill., Hawaiian Fungi No. 1096.

Spots amphigenous, angular, vein-limited, 1–3 mm., at first dark-brown, becoming gray to white centered; border more or less indefinite. Mycelium internal, regular to irregular, 1.5–7  $\mu$ , subhyaline to hyaline, stromatic mycelium subhyaline to brown, 3.2–9.8  $\mu$ . Conidiophores amphigenous, solitary to loosely to fairly densely tufted, emerging through the stomata or rupturing the epidermis, simple, straight to subflexuous, with a more or less bulbous base, non-stromatic or arising from a loose to fairly compact stroma, pale-brown to deep-brown, 25–220  $\times$  3.5–5  $\mu$ , continuous, or 1–5-septate; conidial scars distinct, mostly over 2  $\mu$  in diameter, somewhat laterally displaced or prominently shouldered. Conidia acicular to slightly acicular-obclavate, pale greenish-yellow, 25–195  $\times$  2.5–3.3  $\times$  1.4–1.8  $\mu$ , 3–15–25-septate.

On leaves of *\*Arctium lappa* L. (wild and cultivated) and *\*Ambrosia trifida* L.

*C. Arcti-ambrosiae* Hals. has not been recorded in the Sylloge Fungorum. This no doubt accounts for the double nomenclature in this case.

Specimens examined: *C. Arcti-ambrosiae* Hals., Seym. & Earle, Ec. Fungi 296 (type), New Brunswick, N. J., and No. 297, New Brunswick, N. J. *C. Arctii* Stev., Herb. Univ. Ill., Hawaiian Fungi No. 1096 (type), Kukuihaele.

CERCOSPORA POROPHYLLI Stev. & Moore, Ill. Biol. Mon. 11: 58. 1927.

Type locality: Siquirres, Costa Rica, Herb. Univ. of Ill., Fungi of Costa Rica No. 554.

Spots amphigenous, circular to angular, more or less vein-limited, 1.5–5 mm., above olive-green to dirty-brown, becoming cinereous, olive-green below; border definite, consisting of one or more narrow, raised ridges, smoky-brown, the whole surrounded by a purplish-brown discolored zone. Mycelium internal, hyaline to olive-brown, 2.5–5  $\mu$ . Conidiophores amphigenous, solitary or loosely tufted, rupturing the epidermis or emerging through the stomata, simple or very rarely branched, subflexuous, more or less geniculate, non-stromatic or loosely stromatic, brown, 35–105  $\times$  3.2–5  $\mu$ , 1–2-septate; conidial scars distinct, laterally displaced and somewhat denticulate or shouldered. Conidia at first cylindrical, later acicular-obclavate, subhyaline, 30–70  $\times$  3–4.5  $\times$  2.5–3.5  $\mu$ , somewhat obscurely 1–7-septate.

On leaves of *Porophyllum ruderale* (L.) Cass.

The conidia of the species produce small secondary conidia at the tip or on short lateral projections near the tip.

Specimens examined: Herb. Univ. Ill., Fungi of Costa Rica No. 554 (type), Siquirres.

**Cercospora malayensis** Stev. & Solh. sp. nov.

Type locality: Mt. Maquiling, near Los Baños, Province Laguna, Philippine Islands, Fungi, Malayana No. 120.

Spots in the nature of long, fairly broad, yellow to tan to brown streaks between the major veins of the leaf, frequently extending from the edge of the leaf to the midrib, the tissue shrivelling more or less and eventually dropping out leaving very ragged edges, the conidiophores forming dark-brown, sooty patches here and there on the discolored areas; border indefinite. Mycelium internal, hyaline to yellowish-brown, 1.5–5.5  $\mu$ , up to 11  $\mu$  in the stromata. Conidiophores amphigenous, moderately to compactly tufted, emerging through the stomata or rupturing the epidermis, straight to flexuous, at times somewhat geniculate, simple, arising from a loose to fairly compact stroma, olive-brown to brown with a reddish tint, 25–260  $\times$  3–5  $\mu$ , continuous or 1–8-septate, conidial scars scattered, fairly large, distinct and somewhat shouldered or by lateral displacement becoming somewhat indistinct. Conidia acicular to acicular-obclavate, hyaline, 50–270  $\times$  2.5–4  $\times$  1–2.5  $\mu$ , somewhat obscurely 4–40-septate. [FIG. 6.]

On leaves of *\*Hibiscus esculentus* L.

The specimen examined was referred to *C. Hibisci* Tracy and

Earle by Saccardo. However it is quite distinct from this species as well as from *C. hibiscina* Ellis & Ev. It differs from both of these species in the nature of the effect on the host as well as having simple conidiophores and long, acicular conidia. *C. brachypoda* Speg. has short conidiophores,  $5-10 \times 3 \mu$  and bacilliform conidia,  $30-50 \times 2-2.5 \mu$ . *C. Hibisci-manihotis* P. Henn. has cylindrical, curved conidia,  $20-60 \times 4 \mu$ . These characteristics of the latter species are quite distinct from those of the above described species.

Specimens examined: Herb. Univ. Ill., Fungi Malayana No. 120 (type), Mt. Maquiling, near Los Baños, Philippines.

**CERCOSPORA CITRULLINA** Cooke, Grevillea 12: 31. 1883.—Ellis & Ev. Jour. Myc. 1: 20. 1885.—Sacc. Syll. Fung. 4: 452. 1886.—Atkinson, Jour. Elisha Mitch. Soc. 8: 45. 1891.—Heald & Wolf, Bu. Pl. Ind. Bull. 226: 45. 1912.—Schwarze, N. J. Bull. 313: 132. 1917. *Fig. 795.*

Type locality: South Carolina, Rav. Fungi Am. No. 589.

Spots amphigenous, circular, 0.5–2–5 mm., brown, becoming whitish; border definite, slightly raised, dark-brown to purplish. Mycelium internal and external: external mycelium, regular, fine,  $1.5-2.5 \mu$ , subhyaline; internal mycelium irregular, olivaceous to fairly dark-brown,  $2-6.5 \mu$ . Conidiophores amphigenous, moderately tufted, rupturing the epidermis, simple, straight to flexuous, arising from a small, loose to compact stroma, reddish-brown.  $45-200-385 \times 4-5 \mu$ , 2–15-septate; conidial scars distinct, laterally displaced or shouldered. Conidia acicular, subhyaline,  $75-290 \times 3-4 \times 1.5-2 \mu$ , somewhat faintly 7–35-septate.

On leaves of *\*Citrullus vulgaris* Schrad.

The original description records the conidiophores as being epiphyllous, and pale-olivaceous. While the specimen examined had amphigenous and fairly dark conidiophores it did not appear otherwise to differ from the original description and is no doubt an authentic specimen of the species under which it is recorded.

Specimens examined: Herb. Univ. Ill., No. 5446, J. A. Stevenson, Pueblo Viejo, Porto Rico.

**Cercospora Leonuri** Stev. & Solh. sp. nov.

Type locality: Cartago, Costa Rica, Herb. Univ. Ill., Fungi of Costa Rica, No. 33.

Spots amphigenous, circular to irregular, more or less vein-limited, rarely confluent, 0.5–2.5 mm., brown; border definite, slightly raised, 75–150  $\mu$ , somewhat darker brown than the spot above, same color as the spot below. Mycelium internal and external: external mycelium subhyaline, 2–4.5  $\mu$ ; internal mycelium subhyaline to olivaceous, 3–6.5  $\mu$ . Conidiophores amphigenous, loosely to moderately tufted, emerging through the

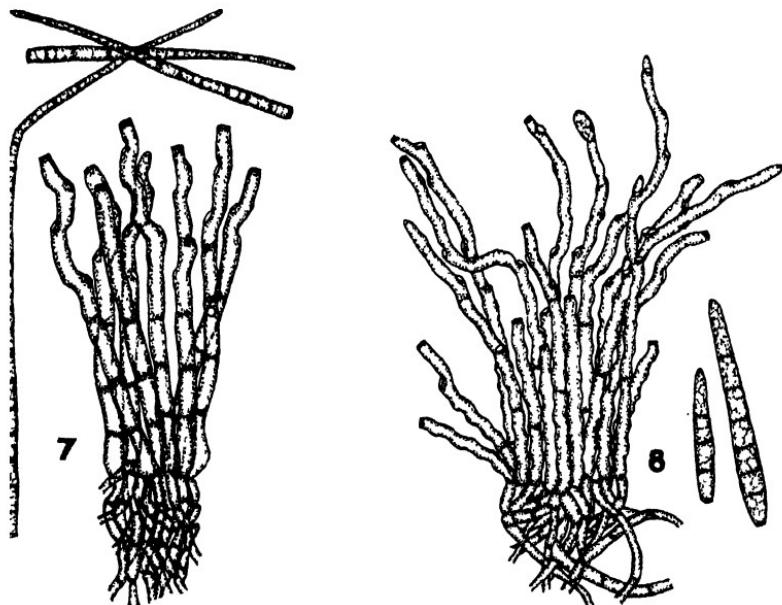


FIG. 7. *Cercospora Leonuri*. Conidial scars over 2  $\mu$ , conidiophores simple, stroma loose to compact, conidia acicular.

FIG. 8. *Cercospora verruculosa*. Conidial scars over 2  $\mu$ , conidiophores simple, stroma loose to compact, conidia abruptly obclavate.

stomata or rupturing the epidermis, simple, straight to subflexuous, more or less geniculate, frequently with bulbous bases, non-stromatic or arising from a small, loose to compact stroma, reddish-brown, 35–110  $\times$  4.5–5.5  $\mu$ , 1–4-septate; conidial scars distinct, laterally displaced or shouldered. Conidia acicular, hyaline or greenish-hyaline, 60–270  $\times$  2.5–4.5  $\times$  1.5–2.5  $\mu$ , somewhat obscurely 7–35-septate. [FIG. 7.]

On leaves of *\*Leonurus cardiaca* L.

Specimens examined: Herb. Univ. Ill., Fungi of Costa Rica No. 33 (type), Cartago.

## SECTION XXIV

Conidial scars distinct, over  $2\ \mu$  in diameter, conidiophores simple, stroma composed of loosely to fairly compactly interwoven hyphae or rarely none produced, conidia abruptly obclavate.

**Cercospora verruculosa** Stev. & Solh. sp. nov.

Type locality: St. Augustine, Trinidad, Herb. Univ. Ill., Fungi of Trinidad No. 829.

Spots amphigenous, angular, more or less vein-limited, up to 1 cm., yellowish to tan to fairly dark-brown above, brown below; border definite, blackish-brown. Mycelium internal, hyaline,  $1.6\text{--}5\ \mu$ , stromatic mycelium straw colored. Conidiophores hypophylloous, moderately to densely tufted, emerging through the stomata and rupturing the adjacent epidermis, straight below, verrucose, flexuous and geniculate above, simple or occasionally with both alternate and opposite branches, arising from a loose to fairly compact stroma, dilute olive-brown,  $50\text{--}250 \times 3.5\text{--}4.5\ \mu$ , continuous or 1-3-septate; conidial scars distinct, shouldered. Conidia abruptly obclavate to obclavate, subhyaline to dilute-yellowish,  $35\text{--}70 \times 5\text{--}8 \times 3.5\text{--}5\ \mu$ , 2-6-septate. [FIG. 8.]

On leaves of *\*Caladium* sp.

Two conidia were observed germinating producing conidiophores directly.

This species is closely allied to *C. Callae* Peck & Cl. from which it differs in the much narrower, longer and verrucose conidiophores. It differs from *C. Caladii* Cooke in its much thicker conidia and from *C. pachyspora* Ellis & Ev. in its septate conidiophores, more slender and more septate conidia.

Specimens examined: Herb. Univ. Ill., Fungi of Trinidad No. 829 (type), St. Augustine.

CERCOSPORA MIKANIACOLA Stev. Trans. Ill. Acad. Sci., 10: 213. 1917.

Type locality: Utuado, Porto Rico, Herb. Univ. Ill., Porto Rican Fungi No. 7923.

Spots amphigenous, circular to somewhat angular, more or less vein-limited, 0.5-10 mm., above sordid-white in center, the spots enlarging by concentric zonation and the white center becoming surrounded by a dark brown zone which in turn is

surrounded by a grayish-brown zone and the whole surrounded by a blackish zone, similar below but the grayish-brown zone is usually not distinct; border more or less indefinite, rarely raised. Mycelium internal and external: external mycelium subhyaline, fine, 1.5–2.5  $\mu$ ; internal mycelium subhyaline to yellowish to olivaceous, 1.5–5  $\mu$ . Conidiophores hypophylloous or rarely also epiphyllous, emerging through the stomata, solitary or loosely tufted, simple, geniculate, flexuous or rigid, non-stromatic, brown with a reddish tinge, 30–225  $\times$  4.5–6.5  $\mu$ , 1–8-septate; conidial scars distinct, mostly scattered, shouldered, or less frequently somewhat laterally displaced. Conidia abruptly obclavate or at times somewhat fusiform, with a long whip-like tip, subhyaline to dilute-yellowish, 34–125–175  $\times$  4–11  $\times$  1–3.5  $\mu$ , 6–13-septate.

On leaves of *\*Mikania* spp.

A single conidium was seen germinating producing a conidiophore from the basal cell. The conidiophore was fairly dark-brown as compared with the subhyaline conidium. This indicates a genetic basis for the color of the conidiophore.

Specimens examined: Herb. Univ. Ill., Porto Rican Fungi No. 7923 (type), Utuado; No. 4700, Maricao; 5083, Aguada; Fungi of British Guiana No. 24, Georgetown.

### SECTION XXX

Conidial scars distinct, over 2  $\mu$  in diameter, conidiophores with alternate branching, stroma composed of loosely to fairly compactly interwoven hyphae or rarely none produced, conidia acicular-obclavate.

**CERCOSPORA ALABAMENSIS** Atkinson, Jour. Elisha Mitch. Soc., **8**: 51. 1891.—Tracy & Earle, Miss. Bull. **38**: 150. 1896.—Underw. & Earle, Ala. Bull. **80**: 141. 1897.—Sacc. Syll. Fung. **10**: 632. 1902.—Stev. Bull. Bernice P. Bishop Mus. **19**: 156. 1925.

Type locality: Alabama.

Spots amphigenous, circular, 1–5 mm., at first brown, becoming dirty-white; border definite, raised, brown, 150–400  $\mu$ . Mycelium internal, irregular, subhyaline to brown, 1.6–8.2  $\mu$ . Conidiophores amphigenous, loosely to fairly compactly tufted, emerging through the stomata or rupturing the epidermis, straight to subflexuous, arising from a loose to compact stroma, dilute reddish-brown, 35–140  $\times$  4–5  $\mu$ , continuous or 1–5-septate,

mostly simple but occasionally monopodially branched, the branches for the most part being short but now and then long and well developed; conidial scars distinct, laterally displaced and somewhat denticulate or shouldered. Conidia acicular, straight or curved, subhyaline,  $50-220 \times 3-4 \times 1.6-2.5 \mu$ , 6-25-septate.

On leaves of *Ipomoea purpurea* Roth., *I. Pes-tigridis* L. and \**I. biloba* Forsk.

The original description describes the border as dark-purple or black and the conidia up to  $250 \mu$ .

This fungus is very closely related to *C. Ipomoeae* Wint. and may prove to be the same or only a variety of it.

Specimens examined: Herb. Univ. Ill., Fungi of Costa Rica No. 617, Puntarenas.

#### SECTION XXXVI

Conidial scars distinct, over  $2 \mu$  in diameter, conidiophores with alternate and opposite branching, stroma composed of loosely to fairly compactly interwoven hyphae or rarely none produced, conidia acicular-obclavate.

CERCOSPORA TECTONIAE Stev. Bull. Bernice P. Bishop Mus., 19: 155. 1925.

Type locality: Oahu, Honolulu, Herb. Univ. Ill., Hawaiian Fungi No. 52.

Spots amphigenous, angular, at times confluent, more or less vein-limited, 1-4 mm., brown to reddish-brown above, brown below, becoming ashen centered: border indefinite or at times definite, slightly raised or not raised, brown. Mycelium internal and external: external mycelium subhyaline to hyaline,  $2-3 \mu$ ; internal mycelium subhyaline to yellowish-brown to olive-brown,  $1.5-6.5 \mu$ . Conidiophores hypophyllous, solitary or loosely tufted, emerging through the stomata or to a slight extent effused on the external mycelium, flexuous, somewhat geniculate, arising from a small stroma, brown with a reddish tinge,  $35-175 \times 3.2-5 \mu$ , 1-7-septate, more or less branched, the branches both alternate and opposite; conidial scars distinct, laterally displaced or shouldered. Conidia acicular, hyaline,  $45-175 \times 3-4 \times 1.5-2 \mu$ , 7-15-septate.

On leaves of \**Tectona grandis* L.

Specimens examined: Herb. Univ. Ill., Hawaiian Fungi No. 52 (type), Oahu: Honolulu, Hillebrand gardens.

**CERCOSPORA FLAGELLARIS** Ellis & Martin, Am. Nat. **16**: 1003. 1882.—Ellis & Ev. Jour. Myc. **1**: 18. 1885.—Sacc. Syll. Fung. **4**: 453. 1886.—Atkinson, Jour. Elisha Mitch. Soc. **8**: 46. 1891.—Stev. Trans. Ill. Acad. **10**: 212. 1917.

Type locality: Eastern United States.

Spots amphigenous, circular to angular, at times confluent, 0.5–10 mm., yellowish-brown above, becoming ashen centered, lighter colored below; border definite, raised, narrow, brown to reddish-brown above, yellowish-brown below. Mycelium internal and external: external mycelium subhyaline, 1–3  $\mu$ ; internal mycelium subhyaline, brown in stroma, 1.5–5  $\mu$ . Conidiophores amphigenous, moderately to fairly compactly tufted, emerging through the stomata, or at times rupturing the epidermis above, subflexuous to flexuous, geniculate, stromatic, dilute-olivaceous to brown, 40–250  $\times$  3.5–5  $\mu$ , 1–10-septate, more or less branched, the branches both opposite and alternate, well developed; conidial scars distinct, laterally displaced or shouldered. Conidia narrowly acicular, bacilliform, greenish, 35–280  $\times$  2.5–3.5  $\times$  1.5–2.5  $\mu$ , 4–20-septate.

On leaves of *\*Phytolacca decandra* L., *\*P. icosandra* L. and *Rivina humilis* L.

Specimens examined: Herb. Univ. Ill. No. 7314, Mt. Carmel, Ill.; ex Herb. Mo. State Univ. No. 20227, Columbia, Mo.; Porto Rican Fungi, No. 2323, Maricao.—Seym. & Earle, Ec. Fungi **360a**, Jamesburg, N. J.; No. **360b**, Blue Ridge, Va.

#### DIDYMARIA CORDA

**Didymaria boringuensis** (Young) Stev. & Solh. comb. nov.

Syn. *Cercospora boringuensis* Young, Mycologia **8**: 45. 1916.

Type locality: Mayaguez, Porto Rico, Herb. Univ. Ill., ex Herb. F. L. Stevens No. 6752.

Spots amphigenous, circular to angular, 1–7 mm., brown; border definite, not raised, brown, darker than spot. Mycelium internal and external: external mycelium subhyaline, 2–3  $\mu$ ; internal mycelium subhyaline to dilute-olivaceous, 1.5–7  $\mu$ . Conidiophores amphigenous but mostly hypophyllous, loosely to moderately tufted, emerging through the stomata, more or less coremioid, simple, flexuous, non-stromatic or loosely stromatic, brown, 50–175  $\times$  2.5–4  $\mu$ , 2–6-septate; conidial scars minute, mostly indistinct, laterally displaced or at times some-

what shouldered. Conidia clavate, straight or curved, dilute-olivaceous,  $25-55 \times 2.5-3.5 \times 4-6.5 \mu$ , 1-7-septate.

On leaves of *\*Calopogonium orthocarpum* Urb.

Specimens examined: Herb. Univ. Ill., ex Herb. F. L. Stevens No. 6752 (type), Mayaguez, Porto Rico.

**Didymaria conjugans** Stev. & Solh. sp. nov.

Type locality: Tumatumari, British Guiana, Herb. Univ. Ill., Fungi of British Guiana No. 54.

Spots amphigenous, irregular, more or less confluent, 2-8 mm., rusty-brown, becoming tan centered; border indefinite. My-

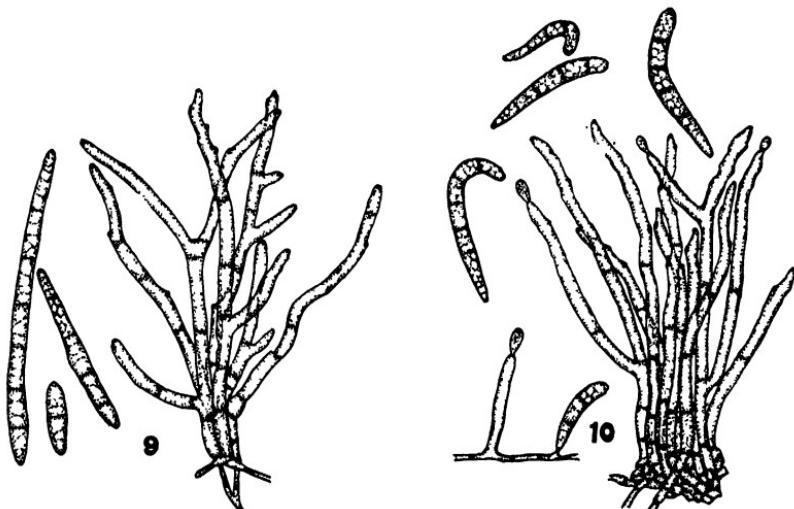


FIG. 9. *Cercospora Cayaponiae*. Conidial scars minute, conidiophores with alternate and opposite branching, stroma none, conidia cylindrical.

FIG. 10. *Didymaria conjugans*.

celium internal and external: external mycelium subhyaline to olivaceous, fine,  $1.2-2 \mu$ ; internal mycelium subhyaline to olive-brown, fine,  $1.5-3.5 \mu$ . Conidiophores amphigenous but mostly hypophyllous, moderately to compactly tufted, emerging through the stomata or effused on the external mycelium, flexuous, arising from a compact to tuberculate stroma, olivaceous,  $35-150 \times 3-4 \mu$ , 2-5-septate, branched, the branches long and well developed, alternate; conidial scars minute, laterally displaced or at times slightly shouldered. Conidia clavate, crooked, olivaceous,  $25-50 \times 2-3.5 \times 4.5-5.5 \mu$ , 1-5-septate. [FIG. 10.]

On leaves of an unknown Legume.

Specimens examined: Herb. Univ. Ill., Fungi of British Guiana No. 54 (type), Tumatumari.

**Ragnhildiana** Solheim, gen. nov.

Conidiophores tufted, emerging through the stomata or rupturing the epidermis, or effused on an external mycelium, simple or monopodially branched, more or less geniculate, straight or flexuous, continuous or septate, arising from a loose to compact or tuberculate stroma, hyaline to dark-brown. Conidia catenate, acrogenous, at times appearing lateral due to further development of the conidiophores, cylindrical, continuous or several-septate, hyaline to dark-brown.

The type species is *Ragnhildiana Agerati* (Stevens) Stevens and Solheim.

This genus is intermediate between *Cladosporium* and *Cercospora*.

**Ragnhildiana Agerati** (Stevens) Stev. & Solh. comb. nov.

Syn. *Cercospora Agerati* Stev. Bull. Bernice P. Bishop Mus., 19: 154. 1925.

Type locality: Hawaii: Kealakekua, Herb. Univ. Ill., Hawaiian Fungi No. 944.

Spots somewhat indefinite, irregular, more or less confluent, vein-limited, yellowish-brown to brown above, smoky-brown below; border indefinite. Mycelium internal and external: external mycelium regular, subhyaline, fine, 2-3  $\mu$ ; internal mycelium fairly regular, hyaline, subhyaline, or brownish in stromata, 1.4-2.5  $\mu$ . Conidiophores amphigenous but mostly hypophyllous, loosely to moderately tufted or effused on the external mycelium, emerging from the stomata, flexuous, arising from a small loose stroma, hyaline, subhyaline to dilute-brown, 25-70  $\times$  3-5  $\mu$ , attenuated toward bases, continuous or 1-4-septate, much branched, the branches of monopodial origin and well developed; conidial scars fairly distinct, laterally displaced or slightly shouldered. Conidia catenate, cylindrical, subhyaline, 15-52  $\times$  2.5-4  $\mu$ , continuous or 1-4-septate. [FIG. 11.]

On leaves of *\*Eupatorium repandum* Willd. (*Ageratum conyzoides*.)

Specimens examined: Herb. Univ. Ill., Hawaiian Fungi No. 944 (type), Kealakekua; No. 750, Wailuku River.

**Ragnhildiana Cyathulae** Stev. & Solh. sp. nov.

Type locality: Coverden, British Guiana, Herb. Univ. Ill., Fungi of British Guiana No. 743.

Spots amphigenous, circular, 0.5–1.5 mm., brown, or black with brown centers above, similar below but appearing olive-brown due to the conidia and conidiophores; border indefinite.

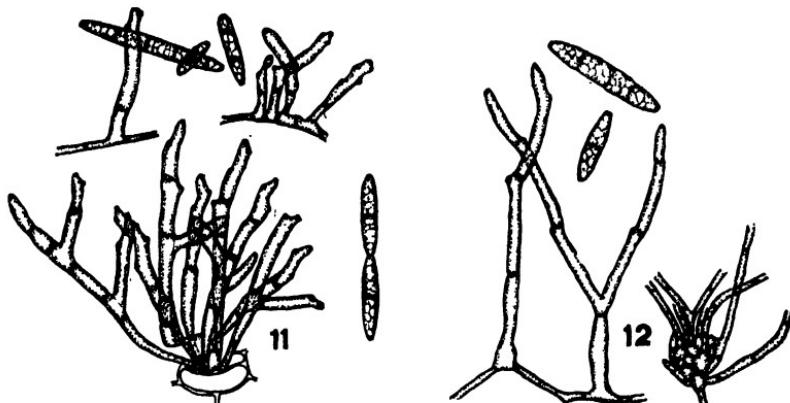


FIG. 11. *Ragnhildiana Agerati*.

FIG. 12. *Ragnhildiana Cyathulae*. To the left, conidiophores and conidia; to the right, internal mycelium with external mycelium arising from it.

Mycelium internal and external: external mycelium arising from the internal and emerging through the stomata, subhyaline to brown,  $1.5\text{--}5 \mu$ ; internal mycelium forming scattered dense mats with connecting hyphae, light smoky to brown,  $2.5\text{--}7 \mu$ . Conidiophores hypophyllous, arising from the external mycelium, creeping, branched, flexuous, brown,  $50\text{--}200 \times 3\text{--}5 \mu$ , 2–6-septate; conidial scars distinct, borne singly at the upper end of the cell from which the sympodial branch arises. Conidia cylindrical, catenulate, subhyaline to smoky,  $15\text{--}45 \times 4\text{--}7 \mu$ , 1–3-septate. [FIG. 12.]

On leaves of *\*Cyathula achyranthoides* Moq.

Specimens examined: Herb. Univ. Ill., Fungi of British Guiana No. 743 (type), Coverden.

**Ragnhildiana gonatoclada** (Sydow) Stev. & Solh. comb. nov.

Syn. *Cercospora gonatoclada* Sydow, Ann. Myc. 23: 425. 1925.

Type locality: La Caja near San Jose.

Spots somewhat indefinite, the conidiophores forming dark circular patches on the lower surface of the leaves, the upper

surface of the leaves becoming light-green to yellowish; border indefinite. Mycelium internal, hyaline to yellow,  $2-3.5\ \mu$ . Conidiophores hypophylloous, covering the whole spot, moderately to fairly densely tufted, emerging through the stomata, straight to subflexuous, more or less geniculate, arising from a loose to fairly compact stroma, smoky-brown,  $30-110 \times 3.5-5.5\ \mu$ , 1-7-septate, branched, the branches alternate and well developed, usually arising near the bases; conidial scars distinct, laterally displaced or shouldered. Conidia cylindrical, catenulate, subhyaline to dilute-brownish,  $25-60 \times 4-5.5\ \mu$ , continuous 1-3-5-septate.

On leaves of *\*Iresine paniculata* (L.) O. and *I. calea* (Iban.) Standl.

Both conidia and conidiophores were observed anastamosing.

This fungus was originally published under *Cercospora Gilbertii* Speg., Trans. Ill. Acad. Sci. 10: 211. 1917. It differs from this species in having branched, septate conidiophores and thicker, catenulate conidia.

Specimens examined: Herb. Univ. Ill., Porto Rican Fungi No. 8286 (type), Bandera.

**Ragnhildiana Manihotis** Stev. & Solh. sp. nov.

Type locality: Penal Settlement, British Guiana, Herb. Univ. Ill., Fungi of British Guiana, No. 683.

Spots amphigenous, scattered, circular to irregular, more or less vein-limited, 0.5-3-6 mm., at first brown, becoming pure white above, similar below but with centers remaining somewhat brownish; border definite, raised, brown,  $150-500\ \mu$ . Mycelium internal and external: external mycelium subhyaline, fine  $1.5-2\ \mu$ ; internal mycelium irregular, subhyaline,  $2-5.5\ \mu$ , stromatic mycelium brownish. Conidiophores amphigenous but mostly hypophylloous, moderately tufted, emerging through the stomata, straight to flexuous, intertwined, geniculate, stromatic, olive-brown to reddish-brown,  $40-200 \times 3.5-5\ \mu$ , 1-6-septate, simple or quite frequently branched, the branches both alternate and opposite, when opposite subtending a conidial scar; conidial scars distinct, shouldered. Conidia catenulate, obclavate to cylindrical, hyaline,  $15-45 \times 4-8 \times 3-4.5\ \mu$ , continuous or 1-3-septate.

On leaves of *\*Manihot utilissima* Pohl.

The following specimens formerly reported from Porto Rico as *C. Henningsii* Allesch. belong under this species:

Santuree, 254; Hormigueras, 223; Bayamen, 3932. One reported from Dos Bocas, 6557 as *C. Cassavae* Ellis & Ev. also belongs here.

Specimens examined: Herb. Univ. Ill., Fungi of British Guiana No. 683 (type), Penal Settlement; Porto Rican Fungi No. 254, Santuree; No. 223, Hormigueras; No. 3932, Bayaman; No. 6577, Dos Bocas; Fungi of Panama No. 1181, Frijoles.

**Ragnhildiana Tremae** Stev. & Solh. sp. nov.

Type locality: St. Clair, Trinidad, Herb. Univ. Ill., Fungi of Trinidad No. 889.

Spots amphigenous, circular to irregular, 3–5 mm., grayish-brown center surrounded by a dark-brown to purplish-brown zone which in turn is surrounded by a zone which is reddish-brown above and brown below; border indefinite. Mycelium internal, hyaline, olivaceous to brown, irregular, 2–4  $\mu$ . Conidiophores hypophyllous, loosely to moderately tufted, emerging through the stomata or rupturing the epidermis, also arising from the trichomes, simple or at times branched, straight, more or less geniculate, stromatic, olivaceous, 20–60  $\times$  3–4.5  $\mu$ , 1–3-septate; conidial scars minute, distinct, laterally displaced or shouldered. Conidia cylindrical, catenulate, dilute olive-green, 25–55  $\times$  2–3.5  $\times$  2–3  $\mu$ , 1–5-septate.

On leaves of *\*Trema micrantha* Blume.

Specimens examined: Herb. Univ. Ill., Fungi of Trinidad, No. 889 (type), St. Clair.

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## NOTES AND BRIEF ARTICLES

Dr. B. O. Dodge has just turned over to the Mycological Department of The New York Botanical Garden one hundred specimens of fungi "Herbarium Mycologicum Romanicum" issued by Professor Dr. Tr. Săvulescu of Roumania. These specimens are unusually well put up with descriptive labels and are a valuable addition to our collection of exsiccati.

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Mr. John A. Stevenson, Mycologist of the Bureau of Plant Industry, Washington, D. C., spent several days at the Garden in March looking over fungi belonging to the family Perisporaceae. Mr. Stevenson formerly spent several years in Porto Rico and is especially interested in this group of fungi, most of which are tropical.

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Dr. Alfred H. Povah has been appointed Park Naturalist of Yellowstone National Park, with headquarters at Mammoth Hot Springs. The other permanent members of the Educational Staff are Dorr G. Yeager, Assistant Park Naturalist, and Miss Herma Albertson, Junior Park Naturalist. The educational program of conducted trail trips, lectures at hotels, lodges and auto camps as well as museum duty will be carried out by this staff assisted by a temporary staff of twenty-two ranger naturalists.

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At the Fifth International Congress held at Cambridge, England, 1930, it was decided by an overwhelming majority to retain the rule adopted at a previous convention (1905) that the publication of names of new groups would be valid only when accompanied by a Latin diagnosis. The date on which the rule was to become effective was changed from January 1, 1908, to January 1, 1932. By a diagnosis is meant not necessarily a long detailed description but a concise statement of the leading diagnostic characters. Contributors to *MYCOLOGIA* are requested to comply with this provision. Each author will be responsible for his own Latin.

Doctor C. H. Kauffman, Emeritus Professor of Botany and Emeritus Director of the University Herbarium of the University of Michigan, died at his home in Ann Arbor, Michigan, the morning of June 14 after a sickness of sixteen months as the result of a paralytic stroke in February 1930. He was sixty-two years of age.

Dr. Kauffman came to the University of Michigan as an Instructor in Botany in 1904. He was made Assistant Professor in 1910, Associate Professor in 1920, Professor in 1923. In 1921 he became Director of the University Herbarium. He was absent on leave from 1917-1919 during which time he served as Pathological Inspector on the Federal Horticultural Board, United States Department of Agriculture.



GIANT ELVELA

# MYCOLOGIA

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## PHOTOGRAPHS AND DESCRIPTIONS OF CUP-FUNGI—XV

### THE GIANT ELVELA<sup>1</sup>

FRED J. SEAVER

(WITH PLATE 29)

In April, 1931, the writer received from Dr. Leva B. Walker, of the University of Nebraska, an unusual specimen of *Elvela* collected by Dr. W. E. Bruner of Baldwin, Kansas. As pointed out by Dr. Walker, this specimen is like the illustration in Hard's mushroom book under the name of *Gyromitra esculenta*. His species, however, was wrongly named and the illustration (figure 418) was referred by the writer in North American Cup-fungi to *Elvela caroliniana* of which the European form *Elvela Gigas* Krombholz was cited as a synonym.

There is a great deal of speculation as to what the Krombholz plant really was. The specific name *Gigas* indicates that it was a giant form. In the description it is stated that it was 4 to 12 inches high and broad. The spores are described as large and ellipsoid. The term large here is doubtless used in contrast with those of *Elvela esculenta*, which was listed in the same work and in which the spores are very much smaller. From the colored illustration accompanying his work the pileus is noted to be chestnut-brown.

Boudier's illustration of the Krombholz species shows the spores to be more fusoid than those of our specimens. Also,

<sup>1</sup> This paper is supplementary to The North American Cup-fungi (*Operculatæ*), which was published by the author and issued December, 1928.

[Mycologia for Sept.-Oct. (23: 313-407) was issued Sept. 1, 1931.]

the convolutions of the pileus seem to be less intricate. Our plant agrees fairly well with Krombholz's description and for the time being it is referred to that species. For a complete synonymy and description see North American Cup-fungi (Operculates), page 253. The specimen shown in accompanying illustration was eight inches high. The pileus was five inches broad and chestnut-brown. The stem was three and one-half inches at the base and two and one-half inches at the narrowest point. The spores are ellipsoid, and contain one large oil-drop with usually several smaller ones and at maturity are rough and  $12-14 \times 30-36 \mu$ . On close inspection they are found to be delicately reticulated. In a later letter we learned that one of the specimens collected by Dr. Bruner was ten and one-half inches high, the largest record received to date.

The writer would be glad to receive any specimens or photographs of this and related forms looking toward a revision and extension of our knowledge of the Elvelaceae. The species of this family are so variable in form that photographic records should accompany specimens whenever it is possible to get them.

THE NEW YORK BOTANICAL GARDEN,  
BRONX, NEW YORK CITY

# STUDIES ON THE MORPHOLOGY AND DEVELOPMENT OF AN INSECT-DESTROYING FUNGUS, ENTOMOPHTHORA SPHAEROSPERMA<sup>1</sup>

W.M. H. SAWYER, JR.

(WITH PLATES 30 AND 31 AND 1 TEXT FIGURE)

## INTRODUCTION

Few entomogenous fungi have received more attention, either from mycologists or from entomologists, than *Entomophthora sphaerosperma*; nevertheless, the supposedly obligate parasitism of this species has presented difficulty to its complete study. The writer's success in cultivating the fungus on artificial media has made possible the detailed observation of all phases in its life cycle, and the results are presented in this paper.

## TAXONOMIC HISTORY

*Entomophthora sphaerosperma* was named by Fresenius (6) in 1856, and in 1858 he published a brief description (7), based mainly upon the size and appearance of the resting spores. It is probable that Fresenius did not observe the conidial stage, for although he mentioned another type of spore, his figures indicate that these spores and the mycelium which bore them belonged to some Hyphomycete. The host upon which Fresenius found the fungus was the "cabbage worm," the larval stage of *Pieris Brassicae*.

Brefeld, in a series of papers (2), (3), (4) from 1870 to 1881, described a fungus, also parasitic upon larvae of *Pieris Brassicae*, which he first (1870) called *Empusa radicans*, and later (1881) named *Entomophthora radicans*, because of its branched conidio-phores.

Cohn (5) in 1870 created the genus *Tarichium*, based upon the

<sup>1</sup> Contribution from the Laboratories of Cryptogamic Botany, Harvard University, No. 104.

characteristics of the resting spores, and he changed Fresenius' name *Entomophthora sphaerosperma* to *Tarichium sphaerospermum*. Cohn, moreover, pointed out that Fresenius' *Entomophthora sphaerosperma* and Brefeld's *Empusa radicans* occurred upon the same host and usually at the same time of year, in late summer. He did not say, however, that the species of Fresenius and of Brefeld were identical. It remained for Sorokin (14) in 1883 to state this point clearly; Nowakowski (10) seems to have failed to note the identity of the two species, for he described the fungus under Brefeld's name of *Entomophthora radicans*. The fungus was first recorded in this country on the clover weevil, *Hypera punctata* (*Phytonomus punctatus*) by Arthur (1), who called it *Entomophthora Phytonomi*; Thaxter (16) subsequently showed that this parasite was identical with *Entomophthora sphaerosperma*. The fungus has since been observed on a wide variety of insects, but because of the early work of Fresenius and Brefeld, the larva of *Pieris Brassicae* is still the most generally known host, especially in Europe.

The writer first observed the fungus in 1923 upon larvae of *Rhopobota vacciniana* Packard, the "black-headed fireworm" that attacks cranberry foliage and fruit; this insect is general in its distribution in both Europe and America, wherever cranberries (*Vaccinium macrocarpon*) occur, but has never previously been reported as a host of this *Entomophthora*. It was from the larvae of this insect that the fungus now in culture was obtained in 1927.

#### TECHNIQUE

A description of the methods employed for growing certain entomogenous species of the Entomophthoraceae in artificial culture has already been given by the writer in an earlier paper (13) and this discussion will be limited to a brief review.

*Entomophthora sphaerosperma* was first grown on flesh of the common herring or alewife (*Pomolobus pseudoharengus* Wilson) after attempts to cultivate the fungus on nutrient agar and other standard media had failed. The herring was autoclaved, and then inoculated with fresh conidia that had been collected on a sterile glass plate, inverted over an infected larva in a moist chamber; these conidia germinated readily, and gave rise to a

pure culture of the fungus. Cultures have also been made by transferring bits of the fungus from an infected insect directly to the culture medium; but despite the utmost precaution, such cultures are always contaminated with bacteria, and a laborious series of transfers is necessary before a pure culture can be obtained.

Subsequently the fungus has been grown on about forty different media, of which swordfish (*Xiphias gladius* Linnaeus) and potato have proved to be the two most useful. Preparation is simple; the substance to be used is cut to fit the tube or flask, sterilized for fifteen minutes at ten pounds' pressure, and inoculated. Potato and swordfish need no adjustment for acidity, for their H-ion concentration is within the range most favorable for growth (pH 6.4 to pH 6.8).

*Entomophthora sphaerosperma* grows best at temperatures of 18° C. to 21° C., but will grow well, though more slowly, at lower temperatures. Stock cultures can be kept in the ice-box for at least two months without transfer, and freezing will not injure the fungus; indeed, it stimulates the germination of hyphal bodies. At ordinary temperatures the fungus does not form resting spores on most media; however, resting spores may be readily obtained in great numbers on autoclaved egg yolk.

The material for the following study was obtained from pure cultures. Careful comparison at different stages has shown the fungus to be identical with corresponding stages of *Entomophthora sphaerosperma* on its insect host.

#### MORPHOLOGY AND DEVELOPMENT

The stages in the life-cycle of *Entomophthora sphaerosperma* will be discussed in the order of their developmental sequence.

1. *Conidia.* The conidia are narrowly elliptical, with rounded apex and a tapering base, which is encircled by a barely perceptible collar, marking the ring of attachment to the conidio-phore (PLATE 30, FIGS. 4-11). Average measurements, which are identical for spores produced on the natural host and upon artificial media, are 22  $\mu$  by 7  $\mu$ . A one-layered membrane, thinnest over the basal portion, encloses the spore. The apex is often crowned by a transparent gelatinous cap, which is more

readily apparent after immersion in weak potassium hydroxide solution, followed by staining (PLATE 30, FIGS. 9-11). This cap, though closely adherent, is not an integral portion of the spore membrane, for it can be displaced by rough handling (PLATE 30, FIG. 10), and its staining reaction is sharply differentiated from that of the underlying conidial membrane. Such a modification of the conidial wall has rarely been observed among members of the Entomophthoraceae, although it has long been recognized that certain species (*Empusa Muscae* for example) have protoplasm adherent to the exterior of the spore, due to the ejection of the contents of the conidiophore when the spore is shot off. Doubtless both types of material, though of very different origin, aid the spore in adhering to the host. When a conidium is hurled from its conidiophore, its flight is too swift to be accurately observed; but if one collects discharging conidia of *Entomophthora sphaerosperma* on a cover glass placed obliquely to the line of projection, most of the spores will be found with their apices pointed in the direction of flight (the force of impact causes some to spin around on the smooth surface); from this fact it seems reasonable to assume that they do not turn over in the air, but, arrow-like, travel with the apex ahead, in which case the adhesive apical cap is most advantageously placed to anchor the spore when it strikes the host or some other solid object. It is interesting to note that the conidia of an unnamed *Empusa* which the writer has in culture are of the approximately spherical shape characteristic of spores of *Empusa Muscae*, and are also very adhesive, but like the conidia of *Entomophthora sphaerosperma*, no protoplasm adheres to their exterior nor do they possess the gelatinous cap so evident on the spores of the latter species. It will be interesting to learn, when further study has disclosed the condition in other species, whether this structure has any taxonomic significance, as indeed may be the case if its occurrence is restricted to conidia of species with branched conidiophores and elliptical spores.

The protoplasm is evenly and finely granular, and non-vacuolate in newly formed spores; vacuoles begin to form, however, as soon as the spores have access to abundant moisture. A single nucleus, circular or oval in outline, 3 to 4  $\mu$  in diameter,

and centrally located, is always present. Its outline may occasionally be seen in fresh conidia, but details of structure are not visible until the nucleus is stained.

A. FORMATION AND DISCHARGE. The process of conidial formation and projection in the Entomophthoraceae is of great interest. The following description is based upon studies of small portions of a culture on potato, which were transferred to a cover glass, and the latter then inverted and sealed over a Ward cell. A very small drop of water was sealed in the tip of each arm of the cell; this method obviated the formation of a moisture film on the cover glass, a difficulty encountered with Van Tieghem cells. When the atmospheric humidity in the glass chamber attained equilibrium, the fungus continued to form conidia under what may reasonably be supposed were favorable and practically normal conditions. Observations were made and drawings outlined with a camera lucida at a magnification of seven hundred and twenty diameters.

Mature conidiophores are digitately branched at the distal end. These tips, to a greater extent than the rest of the conidiophore, are distended with dense, finely granular protoplasm, in which are occasional small vacuoles (PLATE 30, FIGS. 2, 3); numerous nuclei also occur, either linearly arranged or obliquely placed in pairs. Each branch is partitioned by one or more cross walls, most frequently by a single septum near the tip, and the terminal portion thus cut off is usually, but not always, uninucleate.

The conidium first appears as a small translucent bud upon the blunt end of a branch; protoplasm flows in from the conidiophore, and ten or fifteen minutes after its initiation the conidium has attained more than half its mature size (PLATE 30, FIGS. 14, 15). A definite sequence of events now follows: the nucleus passes into the spore, the conidial membrane thickens, and the circle of attachment between spore and conidiophore becomes differentiated as a short collar (the basidium of some writers), narrower than the rest of the conidiophore. A thin wall forms across the base of the spore, separating its contents from the conidiophore, which now also forms a limiting cross wall in close apposition to the conidial cross wall. Until this occurs, the pro-

toplasm has had free ingress to the enlarging spore; with the closing of this entrance, pressure within the conidiophore is increased, so that the two cross walls are forced convexly into the spore, and the attachment between conidium and conidiophore is ruptured in a ring around the base of the former. The spore is at once violently discharged, so swiftly that its flight, as seen through the microscope, is like a flash of light. Obviously, the flight is too rapid for details to be observed; but as the conidium comes to rest upon the cover glass, it can be seen that the end which was attached is now everted to form the tapering base of the spore, marked, where it was torn loose from its attachment, by a roughened external ring. Of the several forces operative in the discharge of the conidium, the writer believes that the most potent is the recoil of this basal wall, which acts as a spring to push the spore into space as soon as the circle of attachment to the conidiophore is broken by the pressure within the latter.

The behavior of the conidiophore and its content varies considerably in the period concurrent with, and immediately after, the projection of the spore. Usually the sudden release of pressure causes the thin elastic membrane across the neck of the conidiophore to stretch abruptly, and as rapidly to contract, so that the protoplasm rushes forth to form momentarily a terminal globule, which as quickly retreats within the conidiophore; the latter is thus left turgid and normal in appearance except for a slight infolding at the tip (PLATE 30, FIGS. 24, 25). Sometimes the conidiophore and its terminal membrane are so stretched that some of the elasticity is lost, and only partial withdrawal of the conidiophore content is effected. Again, there may be a weak discharge of the conidium with no emergence of protoplasm from the conidiophore.

The writer has observed many conidiophores in the act of spore discharge, and in no case has there been an actual loss of content; in those cases in which the protoplasm emerges, whether abruptly or slowly, the terminal membrane of the conidiophore, although it may stretch greatly, always remains unbroken, and the content is completely or partially withdrawn.

Although processes in the normal formation of the conidia

may best be observed in highly humid air, as outlined above, study of conidiophores mounted in water is also helpful. In plate 30, figure 19 may be seen a nearly full-grown conidium in which the basal cross wall is beginning to form; the latter appears in optical section as a shelf-like projection from either side. Centripetal development of this wall results in a complete separation of spore content from conidiophore protoplasm, as seen in figure 20. Olive (11) has described the formation of cross walls in the Entomophthoraceae by centripetal development, but the cleavage furrow which he emphasized was not observed in this case. In figure 21, the protoplasmic pressure within the conidiophore has pushed the walls convexly into the spore; and in figure 22, two walls, the basal septum of the spore and the terminal septum of the conidiophore, may be plainly seen. Sections of fixed and stained material have also been observed, but were found inferior to fresh, living material.

Investigators have expressed different opinions regarding conidial formation in the Entomophthoraceae. Thus Thaxter (l.c., pp. 143-144) regards the conidium as a one-spored sporangium; the wall of the protuberant conidiophore tip thus functions as a sporangial wall, within which the single spore differentiates, and the terminal membrane of the conidiophore becomes a true columella. All attempts by the writer to demonstrate a double character of the conidial wall have failed, and he believes that the *Entomophthora* conidium is not a sporangiospore. Both Thaxter (l.c.) and Vuillemin (17), however, have described conditions in which an outer (sporangial) wall was separated and plainly distinguishable from the spore wall. The former has described and figured (l.c., figs. 320, 321) conidia of *Empusa sepulchralis* in which absorption of water had caused a wide separation of the two walls, and the spore is apparently floating freely within a sporangium; see text figure B, 6, 7. Vuillemin (l.c.) in describing a new species, *Entomophthora gloeospora*, placed considerable emphasis on a condition (TEXT FIGURE D, 1-3) observed during spore formation as well as in the ripe spore, in which there is a distinct gelatinous layer between the outer surface of the conidium proper and the episporule (sporangial wall). He distinctly states that this is not a gelatini-

zation of the spore wall, and that the episporic is a separate membrane. On the basis of this gelatinous coat and its limiting membrane, Vuillemin would regard *Entomophthora gloeospora* as "a transition form between the clearly exogenous conidia of the Entomophthoraceae and the sporangia of the Mucorineae."

In conidia of *Entomophthora sphaerosperma* treated with soap solution (TEXT FIGURE E, 1-3) and occasionally even in untreated spores, the writer has observed a condition strikingly similar to the conditions figured by Thaxter and Vuillemin, as described above. In this case, however, the enveloping substance is not a sporangial wall, for it usually does not completely enclose the spore; and furthermore, it sometimes does not occur at all. Moreover, the writer has never been able to observe this substance while the conidium is still attached to the conidiophore. In the case of *Entomophthora sphaerosperma*, at least, this spore envelope is a much swollen condition of the gelatinous apical cap described earlier in this paper. The conidia of all the Entomophthoraceae are markedly adhesive, and it is probable that in the presence of slight moisture there occurs a thin and usually imperceptible layer of this gelatinous material on the conidial surface, which in certain species occasionally becomes developed in sufficient amount to become readily visible.

In the matter of conidial discharge also there has been some difference of opinion. Brefeld (3), for example, who has also described the discharge of conidia of *Entomophthora sphaerosperma*, and whose observations are diagrammatically represented in text figure C, 1-3, states that there is a single septum, the basal membrane of the conidium, which is at first pushed convexly into the conidiophore and later arches out into the conidium. When the spore is freed from the conidiophore by circumferential rupture at its base, it is violently discharged, together with the content of the conidiophore; thus the spore becomes coated with protoplasm, which can be seen clinging to the conidial surface in a freshly shot-off spore. The writer has already made it clear that he has never seen an actual loss of protoplasm from the conidiophore; however, he has also emphasized the differences in behavior exhibited by the conidiophore and its content after spore discharge, and it is entirely possible

that under certain conditions the conidiophore membrane may burst and the entire content be discharged, in which case the spore might become coated with protoplasm and the conidiophore left in the shriveled condition which Brefeld describes. Indeed, it is very probable that the widely different details of spore discharge described for different species of the Entomophthoraceae are largely due to different conditions under which they were observed, rather than to errors in observation or inherent differences in the fungi.

*B. GERMINATION.* Conidia vary in their manner of germination. In water or in humid air a stout germ tube is usually formed (PLATE 30, FIGS. 27-31; 35, 36); sometimes, however, the tube grows vertically and functions as a conidiophore, at whose tip a secondary conidium is formed, precisely like its antecedent except for a slight diminution in size (PLATE 30, FIGS. 32-34).

The secondary conidium is abstricted in the usual manner and may likewise give rise to a tertiary conidium, and so on, until vitality is exhausted or, perchance, an opportunity is afforded for infection. Sometimes the secondary conidium is produced at the tip of a long and extraordinarily thin germ tube (PLATE 30, FIGS. 38-40). This capillary type of conidiophore has been figured by Thaxter (l.c.) for six species including *Entomophthora sphaerosperma*, and seems to belong especially to those forms with relatively small and elongate conidia of the *Entomophthora sphaerosperma* type.

Germination begins with a slight outward bulging of the conidium at any point on its surface, and rapidly proceeds to tube formation. The tube wall differs from the spore wall only in being slightly thinner. The protoplasm, too, is unchanged, except for more vacuolation. The nucleus does not divide until it has migrated into the tube, and, in the cases observed, when a secondary spore is to be formed, the nucleus does not divide at all. Thus the secondary spore has the same nucleus and cytoplasm as did the primary conidium, and it is probable that this condition holds throughout the entire series of secondary spores formed.

When germination takes place in water or air, no food is avail-

able and thus it follows that as the germ tube elongates beyond the expansibility of its protoplasmic content, the latter is carried along in the advancing tip, leaving a vacant area behind. This retreating protoplasm may form a cross wall at its rear and again withdraw, and so a series of empty compartments may occur in the germ tube behind the relatively short terminal space filled with protoplasm (PLATE 30, FIG. 37).

A relative humidity of at least 70 per cent is essential to germination, and the most favorable temperature is near 20° C., when conidia will send forth germ tubes two hours after formation. Spores will not germinate at temperatures of 26° C. or above. Low temperatures retard, but do not prevent, germination; for, as explained in a previous paper (l.c.), conidia remain dormant in ice for at least a week and then germinate readily upon return to favorable temperatures. It is of some interest to note that low temperature (8° C.) during germination results in the formation chiefly of secondary conidia on capillary conidiophores; that this is an effect of low temperature during, and not previous to, germination is shown by the fact that spores which have been frozen and then brought to a temperature near 20° C. form the usual germ tubes, instead of capillary conidiophores.

Light also affects germination. In darkness more conidia germinate than in light and relatively fewer capillary conidiophores occur. The writer has noted that conidia, unless rendered dormant by low temperature as noted above, must germinate at once after their formation or else die. This observation is not in agreement with Brefeld's (4) statement that conidia of this species remain viable for eight days after production.

2. *Mycelium*. Upon proper nutrient media, germ tubes may continue to develop, building up an extensive, branched mycelium (PLATE 31, FIGS. 4, 5). The hyphal walls are thin and the protoplasm finely and evenly granular, with occasional vacuoles. Cross walls do not normally occur in the hyphae unless the latter are about to segment into hyphal bodies, but it has been found in artificial culture that their production may be induced by depletion of the food supply. Nuclei are numerous. Frequently they are evenly spaced in linear sequence; often, however, they occur in pairs, in the same oblique position in which division occurs.

3. *Hyphal bodies.* The mature mycelium segments into the characteristic short individual pieces named by Thaxter *hyphal bodies*. Stages in their formation, as observed by the writer, are as follows: a delicate cross wall begins at the periphery of the hypha and develops centripetally. This wall is thin, single, and unaccompanied by the cleavage furrow described by Olive (l.c.). Usually it develops along the border of a large vacuole, which thereby becomes flattened transversely to the hypha (PLATE 31, FIGS. 7, 8); wall formation through a vacuole has, however, also been observed. The protoplasm then cleaves away from one side of the completed cross wall and the tubular wall of the hypha breaks (PLATE 31, FIG. 9), thus completing the segmentation. It is noteworthy that the transverse wall does not appear to split, but the protoplasm withdraws slightly from one side, so that after the hyphal pieces separate, the protoplasm is restrained in one segment by the transverse wall and in the opposed end of the adjacent segment by the plasma membrane, which may subsequently become slightly thickened. The writer has also seen infected insect larvae in which regions of the invading mycelium had become much attenuated (PLATE 31, FIG. 5) and it is possible that hyphal bodies may sometimes arise through completion of such constrictions.

Old hyphal bodies are prone to form short branches and twist into irregular shapes, while the content becomes less vacuolate, condensed, and coarsely granular (PLATE 31, FIGS. 12-14). Spherical and ovoid forms, often with one or more large central vacuoles (PLATE 31, FIGS. 15-17), consistently occur in old cultures on swordfish. Indeed, the hyphal bodies show all stages in variation between mycelial structures and spherical, spore-like bodies.

4. *Rhizoids.* *Rhopobota* larvae infected with *Entomophthora sphaerosperma* show a remarkable indifference to the progress of the disease, and death does not occur until most of the body tissues are in a marked state of disintegration. About the time the insect dies, some of the hyphae in the thoracic region aggregate and push through the ventral surface of the host as stout tubular structures, which attach the host firmly to the substratum; these are the rhizoids (PLATE 31, FIG. 40) and con-

stitute the only phase in the life cycle of this fungus which is not developed upon artificial culture media.

Each rhizoid consists of many straight, parallel, unbranched hyphae; the presence of bubbles along the lumen when the rhizoid is immersed in water shows clearly that these hyphae are arranged in tubular formation. Where the distal end comes in contact with a solid surface, the individual hyphal ends fan out into a funnel-like, closely appressed mouth, which anchors the insect securely to the substratum (PLATE 31, FIG. 40). For some reason, perhaps because internal development is more advanced in this region, the rhizoids always appear first (in varying numbers) between the thoracic appendages. Their appearance is indicative of the completion of vegetative growth within the host, and this phase is soon followed by conidiophores or by the internal development of resting spores.

*5. Conidiophores.* The conidiophores branch from the hyphal bodies, or, if they develop early, from unsegmented hyphae. The protoplasm often shows streaming movements; nuclear divisions are frequent and nuclei are comparatively abundant. Growth is rapid and the conidiophore may attain full development and begin to liberate ripe conidia within five hours after its initiation.

The writer has not attempted to study the cytology of this species in detail; the frequent nuclear divisions in the conidiophores do, however, make the latter an especially favorable phase in which to observe mitotic behavior, and such details as have been encountered in the general study of the conidiophores will be described at this point.

The resting nuclei are 3 to 4  $\mu$  in diameter. They have a well-defined enveloping membrane and dense, finely granular content. The deep-staining reaction of the granules indicates that they are chromatin; no achromatin network was observed. One to several nucleoli occur, two being the usual number. The nuclear structures stain very well with Haidenhain's haematoxylin; after considerable differentiation in iron-alum solution, the nuclear membrane, chromatin granules, and nucleoli stand out in deep black against the light gray of the surrounding cytoplasm (PLATE 31, FIG. 18).

A definite spireme could not be observed in the prophase; instead, the chromatin aggregates into larger granules to form the individual chromosomes (PLATE 31, FIGS. 35-37), whose number appears to be twelve or more. The nuclear membrane persists until division is completed and the mitotic figures are therefore intranuclear. The chromosomes crowd together in the center of the nucleus into a dense mass in which the individual units are not entirely discernible, and numerous delicate, but well-defined spindle fibres appear (PLATE 31, FIG. 32). The mass of chromosomes now separates into two groups, which begin their migration to the opposite nuclear poles (PLATE 31, FIG. 33). In plate 31, figure 34, may be seen a pair of nuclei that have developed simultaneously; it will be noted that although this is a telophase stage, the nuclear membrane is still intact. This habit of simultaneous division of paired nuclei is of too frequent occurrence to be accidental. Stages in the reconstruction of the resting nucleus were not observed. These steps in mitosis as seen by the writer show close similarity to those described by Riddle (12) for nuclear division in *Entomophthora americana*, with, however, one outstanding difference: the nucleolus in *Entomophthora sphaerosperma* does not become one of the chromosomes, but remains clearly distinguishable from them (PLATE 31, FIG. 37).

Following this initial period of active protoplasmic increase and nuclear multiplication, the nuclei become more widely spaced and the cytoplasm becomes less dense and more vacuolate. From these facts it would appear that the later development of the conidiophore and its conidium is largely dependent on absorption of water.

The conidiophores have a tendency, both in culture and on the host, to aggregate in clumps (PLATE 30, FIG. 1), and although their tops may coalesce to form an uninterrupted surface, these groups retain their individuality throughout the several hours (or days, if environmental conditions are favorable) that they persist. Further details of conidiophore structure have already been described under the discussion of conidia. Sterile conidiophores, interspersed with normal ones, have been noted in some cases (PLATE 30, FIG. 41) upon insects, but never in culture.

6. *Resting spores.* Hyphal bodies may form resting spores, instead of conidiophores; their simultaneous appearance is rare in the insect host, but may be easily brought about in culture by the use of suitable media, such as yolk of egg. The resting spore begins as a protuberance from the end or side of the hyphal body (PLATE 31, FIGS. 20-28), into which cytoplasm and nuclei migrate, leaving the hyphal body partially or completely emptied of its content; usually cross walls are formed behind the retreating protoplasm, as already described in the case of conidial germ tubes. The number of nuclei varies; usually from fifteen to twenty enter each spore. Sections of mature resting spores show as many nuclei, on the average, as do young spores, and it may therefore be assumed that no nuclear fusion takes place, at least during the formation of the spore; Riddle and others have stated for another species that no fusion had occurred in resting spores several weeks, or even months, old.

After the protoplasm has entered the young spore, it is cut off by a thin wall, continuous with the wall of the hyphal body. As the spore matures, this wall thickens somewhat to become the episporule, while the peripheral portion of the enclosed protoplasm secretes the still thicker endospore; the exact steps in the formation of the latter cannot be clearly seen.

Almost as soon as the protoplasm enters the spore from the hyphal body, it becomes denser and less vacuolate, and this condensation continues until, when full grown, the spore is closely packed with coarse granular protoplasm. As the spore ripens, oil globules begin to appear, at first small and numerous, but soon coalescing, until sometimes a single large refractive globule remains, suspended in a protoplasm now clear and homogeneous (PLATE 31, FIGS. 28-31). The development of the resting spore requires about forty-eight hours; final stages in ripening may be hastened by placing the spores in water. The mature spores are spherical and average 15  $\mu$  in diameter.

Digression may be made here to consider briefly certain factors influencing the formation of resting spores. Brefeld, in his researches upon *Entomophthora sphaerosperma*, found, both in nature and in a series of artificial inoculations extending over several weeks, that cabbage-worms containing resting spores, as

compared with those on which conidia were formed, were more numerous as summer progressed into autumn. From this observation he concluded that the resting spore is an adaptation for hibernation, and this theory has led to the general conclusion that the formation of resting spores in the Entomophthoraceae is a result of low temperatures. Thaxter (l.c., p. 150), however, has pointed out that as many infected insects contain resting spores in mid-June as in mid-October, and the writer has found this to be true of *Rhopobota vacciniana*. Indeed, resting spores developed in nearly all infected fireworms kept in the laboratory in early summer, while infected larvae out-of-doors, so far as could be detected, were giving rise only to conidia. The environmental factors that differed in the two cases seemed chiefly to be those of light and temperature, for the experimental larvae in question were in the dark and subjected to a temperature, especially during the night, which was several degrees above the temperature on the cranberry bogs. This led the writer to keep some larvae in the dark and others in light at the same temperature, while two other lots were kept in darkness at different temperatures, one from 12° C. to 16° C., the other from 25° C. to 30° C.

Resting spores were formed in the same number of larvae in light as in darkness, and therefore it would seem that light is not the factor that determines their formation; these observations do not agree with Speare's (15) upon *Entomophthora Pseudococcii*, in which he states that hyphal bodies form conidio-phores when subjected to light, and resting spores when kept in the dark.

The effect of temperature, however, was quite different, for of the larvae kept at 25° C. to 30° C., more than three times as many contained resting spores as did at the lower temperature; it is perhaps noteworthy that at the same time no conidia occurred at 25° C. to 30° C., although they were formed profusely at 12° C. to 16° C. Furthermore, in artificial culture upon potato, it has not been possible to obtain resting spores of *Entomophthora sphaerosperma* at temperatures of 21° C. or below; but when such cultures are kept at 27° C., for forty-eight hours or more, resting spores are formed in profusion.

These facts are strongly indicative that temperatures above the optimum for growth are, contrary to usual opinion, more favorable for the production of resting spores than lower tem-

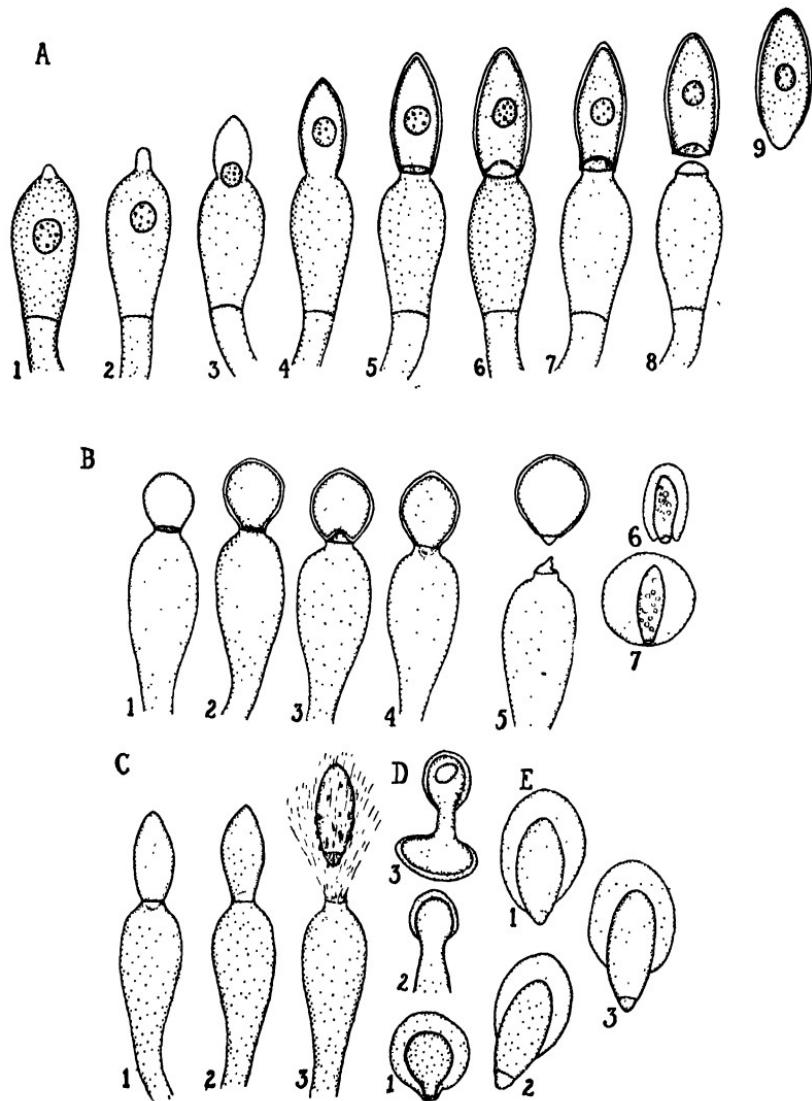


FIG. 1.

peratures. That temperature is not the only factor that may be concerned, however, is indicated by the fact that resting spores of *Entomophthora sphaerosperma* are readily formed upon egg

yolk at 20° C., while, as noted above, a higher temperature is necessary for their production on potato media. Perhaps resting spore formation depends upon a particular mode of protoplasmic activity which in one case is initiated by high temperature, and in the other by chemical "activators" present in egg yolk and absent in potato.

The question of a sexual process in this fungus remains for discussion. In a few species of Entomophthoraceae, the sexual origin of the resting spores has been clearly demonstrated, and beyond doubt they are zygosporcs. In other cases, the spores as obviously develop without conjugation, and are azygospores. In still other species the origin is not clear, but suggests a possibility of a conjugation of hyphal bodies prior to spore formation; *Entomophthora sphaerosperma* belongs among these doubtful forms, for Brefeld (4) described anastomoses of hyphae before the resting spores were formed, but could not associate these unions with individual spores, and Thaxter (l.c.) has described and figured instances that strongly suggest, but do not prove, the conjugation of hyphal bodies in relation to spore formation.

The writer regrets that he is unable to fulfill Lakon's (9) prediction that when *Entomophthora sphaerosperma* could be grown in artificial culture, the sexual origin of its resting spores would be clearly demonstrable. In the great majority of cases, the resting spores are very clearly azygospores (PLATE 31, FIGS. 22-24). Occasionally, however, diligent search will reveal a condition such as is shown in plate 31, figures 27 and 28, which are of the same spore, as seen from opposite sides, which might be interpreted as representing conjugation prior to the formation of the spore. The writer has been totally unable, however, to find any case which could not as readily be interpreted as due to septum formation, a regular incident of spore production in this species. In view of the fact that a thorough examination of countless resting spores has never revealed a clear case of sexuality, and even the doubtful cases are very rare as compared with the definite instances of asexual origin, together with the fact that the structure suggesting conjugation may, in every case seen, as readily be interpreted as an ordinary vegetative cross wall, the writer is of the opinion that the origin of resting spores

in this species is non-sexual, and that they are always azygospores.

Every attempt to bring about germination of the azygospores of *Entomophthora sphaerosperma* has failed, although freezing, drying, heating, treatment with acid, and other measures have been tried. Spores have even been kept out-of-doors for a whole year, under conditions which duplicated those in nature, and although they were frequently examined, germination was never observed.

Gilliatt (8) has recorded germination of the resting spores of *Entomophthora sphaerosperma* in water sixteen days after their suspension in Van Tieghem cells. These experiments were repeated by the writer with azygospores, both from insect larvae and from artificial culture, but germination did not occur. Gilliatt, in those cases where germination took place, neither figured nor described the thick double wall characteristic of true resting spores, and his figures suggest germinating hyphal bodies. Indeed, the writer has yet to be convinced that an authentic case of germination of true resting spores (zygospores or azygospores) has ever been observed in any member of the entomogenous Entomophthoraceae; in no case reported is there definite evidence, either from description or figures, that hyphal bodies were not the structures concerned.

The stages in the life cycle of this fungus as described above (with the exception of the rhizoids) have been studied both in the host and in artificial culture, and have been found to agree in all essential details.

The writer wishes to express to the President and Governing Board of Bates College his appreciation for the leave of absence which made this work possible, and to Professor William H. Weston, Jr., grateful acknowledgment of his never-failing encouragement and inspiration.

#### SUMMARY

1. Cultivation of *Entomophthora sphaerosperma* on artificial media has made a detailed study of this entomogenous fungus possible.

2. The single spores are true conidia, in that they are not formed within a separable sporangium.
3. The forcible projection of the conidium from the conidiophore is due to a definite chain of occurrences, chief among which are swelling of the conidiophore, circumferential rupture of the attachment around its tip, and a recoil of the spore's basal membrane.
4. The conidium possesses a gelatinous apical cap, which aids in its attachment to the host.
5. Conditions most favorable for spore germination are a temperature of 20° C., a relative humidity of 70 per cent or more, and darkness. Conidia are not injured by freezing.
6. Rhizoids are not formed in artificial culture; all other phases of the life cycle are the same in artificial culture as in the insect host.
7. During mitosis, the nuclear membrane persists until the telophase. Definite chromosomes, at least twelve in number, are formed. A well-defined spindle exists and one or more nucleoli are present throughout division, distinct from the chromosomes.
8. Resting spores in this species are formed asexually and are therefore azygospores. Their formation is subject to artificial control in which the determining factors may be either temperature or special artificial media.

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#### EXPLANATION OF PLATES

##### PLATE 30

*Entomophthora sphaerosperma*. Conidia, conidiophores, and hyphal bodies. Fig. 1. A typical group of conidiophores on the surface of the host, showing their interwoven bases, the habit of branching, and conidia. Semi-diagrammatic,  $\times 15$ ; Fig. 2. Two conidiophores, more in detail, separated from a group similar to that shown in fig. 1,  $\times 230$ ; Fig. 3. A young conidiophore. Note the vacuolate cytoplasm, the numerous nuclei, the branches, and a conidium forming at the tip,  $\times 470$ ; Figs. 4-8. Conidia stained with weak safranin-glycerine. The elliptical shape, papillate base, finely reticulate cytoplasm, and single central nucleus with nucleoli and chromatin granules may all be noted,  $\times 830$ ; Figs. 9-11. As above; also a cap-like outer layer of gelatinous material is shown in different stages of development. In fig. 10,

this cap has been displaced to one side,  $\times 830$ ; Figs. 12–18. Successive stages in development of the same conidium at known time intervals: fig. 12 shows the conidium initial just forming at 11:10 p.m.; figs. 13–16 indicate stages in growth at 11:15, 11:20, 11:25, and 11:30 p.m., respectively; fig. 17, at 11:45 p.m., shows the differentiation of a collar-like portion ("basidium") at the top of the conidiophore, the thickening of the wall of the conidium, and the wall formed across its base; fig. 18, at 12:05 a.m., shows the cross wall pushed up into the conidium by pressure of the protoplasm in the conidiophore. This spore was shot off two or three minutes later,  $\times 1000$ ; Figs. 19–22. Conidia in different stages of development, mounted in water and viewed with oil immersion objective. Fig. 19 is of a conidium and conidiophore with a large vacuole in each; note especially the early stage in centripetal development of the conidial cross wall. In fig. 20, this wall is completed. In fig. 21, the wall has become forced convexly into the spore. In fig. 22, two cross walls are present in close apposition, one belonging to the conidiophore and the other to the spore, and both pushed upward; the conidiophore and conidium are thus in the ball-and-socket relation which is attained just before discharge,  $\times 1000$ ; Fig. 23. A conidiophore after discharge of the spore,  $\times 1000$ ; Figs. 24–26. Stages immediately following spore discharge, showing formation of a protoplasmic vesicle and its withdrawal,  $\times 1000$ ; Figs. 27–31. Conidia germinating in water. The large vacuoles and stout germ tubes should be noted,  $\times 1000$ ; Figs. 32–36. Conidia germinating in moist air; figs. 32–34 are stages in the formation of secondary conidia; figs. 35–36 are of conidia forming germ tubes; note the large vacuole in the conidium in fig. 35, and the emptied spore in fig. 36,  $\times 1000$ ; Fig. 37. A condition similar to that in fig. 36, but further advanced; the protoplasm is all in the tip of the germ tube, and the rest of the tube is separated into compartments by cross walls,  $\times 1000$ ; Figs. 38–40. Conidia with capillary conidiophores and secondary conidia,  $\times 1000$ ; Fig. 41. Sterile conidiophores from the midst of normal fertile conidiophores on fireworm,  $\times 470$ ; Figs. 42–43. Young hyphal bodies,  $\times 470$ .

## PLATE 31

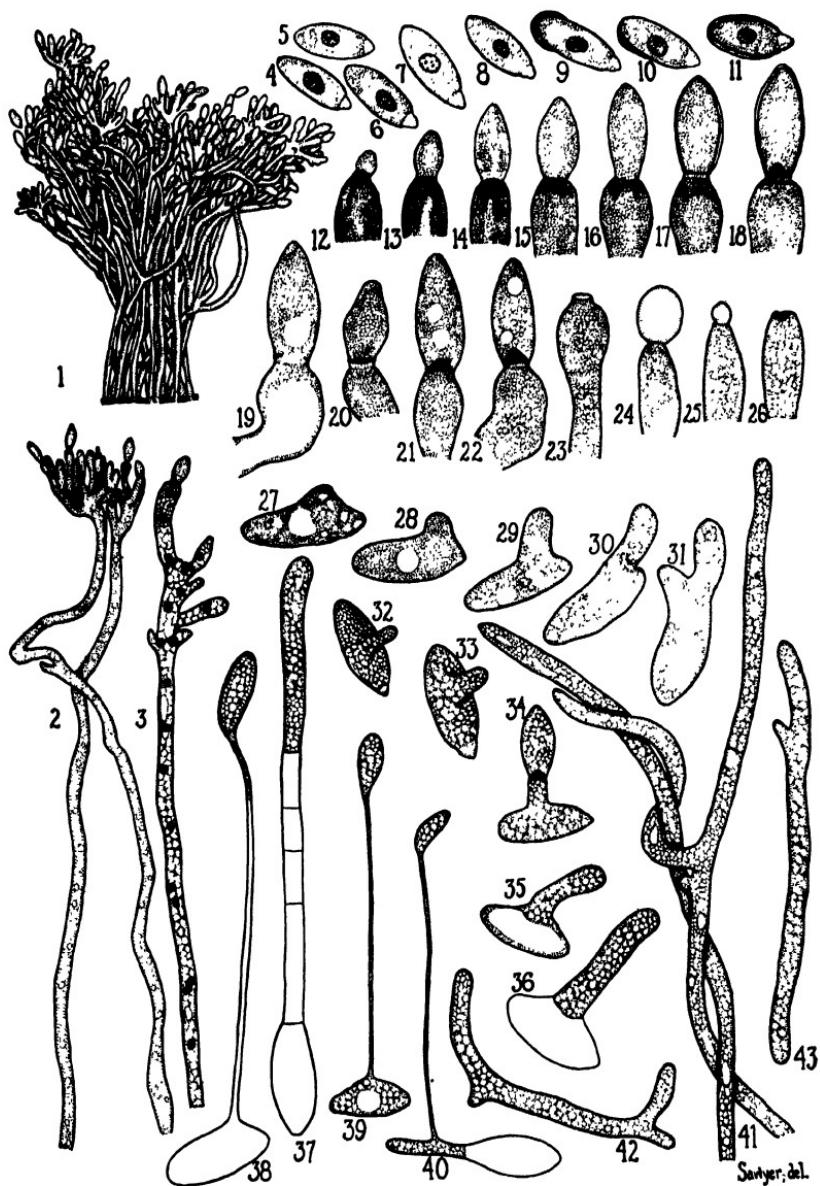
*Entomophthora sphaerosperma*: penetrating germ tubes, mycelium, hyphal bodies, rhizoids, conidiophores, and resting spores.

Figs. 1–3. Conidial germ tubes penetrating the body wall of the host. In figs. 1–2, the conidium has lodged endwise among the cuticular spines and germinated from the apical end, penetrating the primary cuticle and invading the secondary cuticular layer. The germ tube in fig. 2 has branched. In fig. 3, the conidium has germinated in a mass of conidia and debris some distance away and the germ tube has grown to, and penetrated the body wall as far as the hypodermis,  $\times 1000$ ; Fig. 4. A hypha obtained by crushing the body of an artificially inoculated living fireworm. Note the separation in the protoplasm at the bases of the branches, where segmentation into hyphal bodies was probably about to occur,  $\times 230$ ; Fig. 5. Mycelium similarly obtained. The very attenuated condition of two hyphae suggests that hyphal bodies may sometimes be formed by constriction,  $\times 230$ ; Fig. 6. A hyphal body with much convoluted branch,  $\times 230$ ; Figs. 7–10. Stages in segmentation to form hyphal bodies. In fig. 7, a cross wall has formed in

connection with a vacuole; fig. 8, a similar condition which shows the cross wall pressed in upon the vacuole; fig. 9 is of a stage immediately after segmentation of the hypha; the broken ends of the hyphal wall at the point of segmentation, the terminal cross wall in one segment, and the corresponding plasma membrane in the other, may all be seen. Fig. 10 represents a later condition in which one of the segments has branched, forming one of the elbow-shaped hyphal bodies common in these fungi,  $\times 700$ ; Fig. 11. Hyphal body showing nuclei, often in pairs, and the somewhat vacuolate cytoplasm containing deeply staining granules of reserve food material,  $\times 700$ ; Figs. 12-14. Hyphal bodies: fig. 12, from host; figs. 13-14, from potato culture,  $\times 300$ ; Figs. 15-17. Rounded vacuolate hyphal bodies from old swordfish culture,  $\times 300$ ; Fig. 18. Young conidiophore arising as a branch from a small hyphal body. Note the frequent paired arrangement of nuclei,  $\times 700$ ; Fig. 19. Two hyphal bodies overlapping, so that they simulate a condition of conjugation, which, however, is not taking place,  $\times 300$ ; Figs. 20-28. Stages in formation of resting spores. In fig. 20, the spore has begun to form as an enlargement of the end of the hyphal body. The two nuclei in the young spore are merely in contact, not fusing,  $\times 700$ . Figs. 21-23 show resting spores arising terminally; figs. 24-25, laterally. Fig. 26 shows a laterally-formed resting spore on an unusually large hyphal body. Note the separation of protoplasm at the base of the spore, preparatory to wall formation. Figs. 27-28 represent opposite sides of a resting spore which might have been formed as a result of conjugation; it is probable, however, that this appearance is due to a cross wall laid down in the single hyphal body from which the spore arose laterally. In fig. 23, oil globules may be noted, present at an unusually early stage in spore development. Also note that it is usual for cross walls to form in the hyphal bodies as the protoplasm migrates into the growing resting spore,  $\times 300$ ; Figs. 29-31. Ripe resting spores from egg yolk culture. In fig. 29 ( $\times 700$ ) the thick double wall and the numerous oil globules suspended in a clear, homogeneous protoplasm may be noted. Figs. 30-31 ( $\times 880$ ) show a condition in which the oil is aggregated into a few large globules; in fig. 31, the point of attachment to the hyphal body may be seen; Figs. 32-37. These represent phases in nuclear behavior during division. Fig. 32 shows a lateral view of a metaphase stage, with a well-defined spindle and the chromosomes closely compacted in the equatorial plate. The cytoplasm contains densely staining food granules. In fig. 33, an anaphase is shown, soon after division of the chromosomes which are too compacted to permit counting. In fig. 34, a later stage, a pair of nuclei have divided simultaneously. Fig. 35 shows nuclei cut somewhat tangentially, and including but a few chromosomes. Figs. 36-37 show more nearly median sections of nuclei in which the chromosomes are distinct, and also, in fig. 37, a large nucleolus, easily distinguishable from the chromosomes in the same nucleus,  $\times 1600$ ; Fig. 38. Penetration of muscle by hyphae. Note attenuation of hypha where the muscle is penetrated and the space formed on either side of the hypha by enzymic dissolution of the muscle tissue,  $\times 300$ ; Fig. 39. Hypha penetrating cell in wall of stomach,  $\times 300$ ; Fig. 40. Rhizoids emerging through body wall in the mid-ventral thoracic region of the host. Note their branching character, their composition of parallel hyphae, the funnel-like holdfast, and their size as compared with the vegetative hyphae protruding from beneath the cuticle,  $\times 50$ .

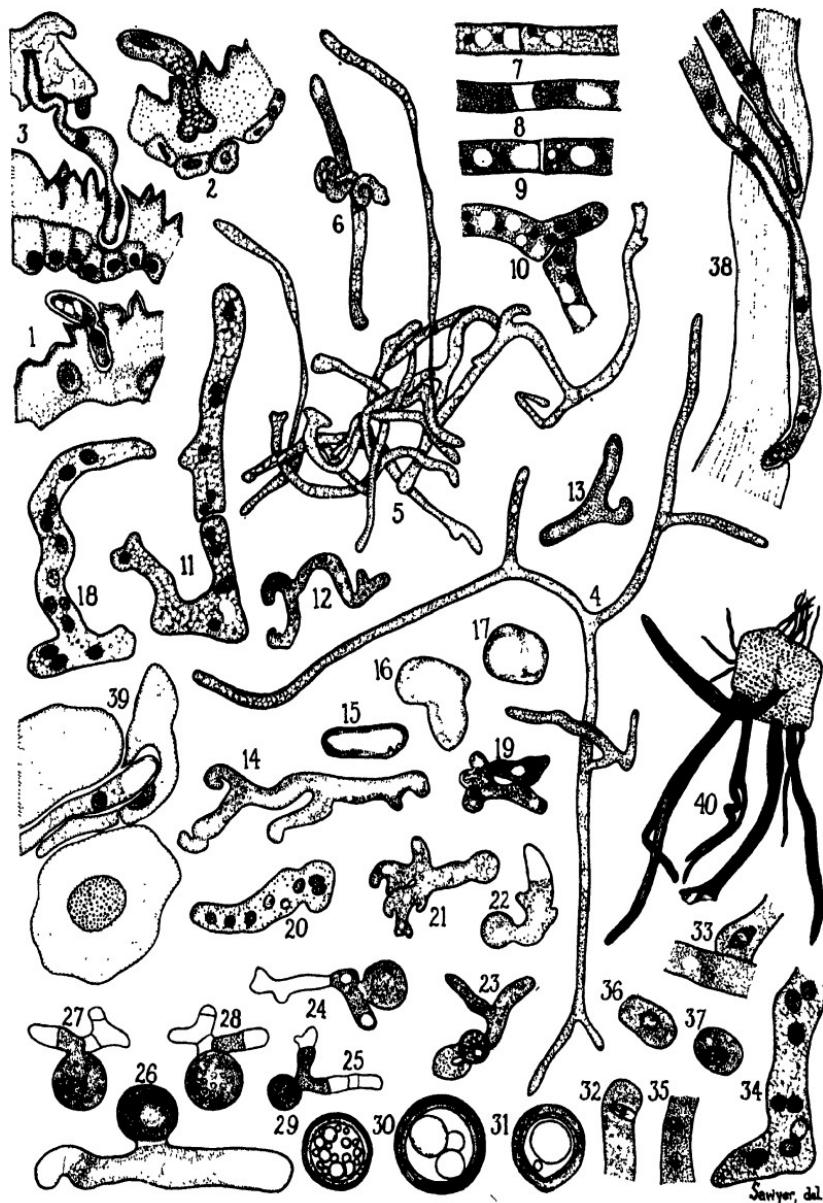
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ENTOMOPHTHORA SPHAEROSPERMA





Sawyer, del.



# PHRAGMIDIUM SPECIES OF NORTH AMERICA: DIFFERENTIAL TELIOSPORE AND AECIAL CHARACTERS<sup>1</sup>

GEORGE B. CUMMINS<sup>2</sup>

(WITH PLATE 32)

Differential teliospore characters of the nineteen species of *Phragmidium* in North America are used herein as a basis for arranging the species occurring on the three host tribes, Potentilleae, Rubeae and Roseae, under the two sections of the genus *Phragmidium*, *Earlea*, characterized by firm non-hygroscopic teliospore pedicels, and *Euphragmidium*, characterized by hygroscopic teliospore pedicels. Aecial characters and teliospore characters are used as bases for keys to the species on each of the three host tribes. For host range and geographic distribution of each species the reader is referred to the North American Flora (3). Measurements are included where they are useful and when pertaining to teliospores do not include the pedicels.

The first comprehensive study of the rust genus *Phragmidium* was published by Dietel (4) in 1904. In this paper he recognized seventeen species in North America and of this number thirteen are accepted as valid today. Arthur (1), in 1906, erected the genus *Earlea* to provide for those species which lacked uredinia in the life-cycle and in 1912 followed the same arrangement in the North American Flora (3). In subsequent works by Sydow (6) and Dietel (5) this separation into two genera was not accepted.

<sup>1</sup> Contribution from the Botany Department, Purdue University Agricultural Experiment Station, Lafayette, Ind.

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## SECTIONS EARLEA AND EUPHRAGMIDIUM BASED ON NATURE OF TELIOSPORE PEDICELS

During the course of this study it has been the writer's privilege to consult the unpublished manuscript of a rust manual prepared by Dr. Arthur in which he divides the genus *Phragmidium* into two sections, *Earlea*, consisting of the species with firm non-hygroscopic teliospore pedicels, and *Euphragmidium*, consisting of the species with hygroscopic pedicels. This subordination of his previously erected genus *Earlea* is based on the fact that the uredinia belong to the same vegetative phase (diploid) as the telia and their presence or absence is considered insufficient basis for generic distinction. The nineteen species to be discussed in this paper will be arranged, with few modifications, in accordance with this scheme.

According to this arrangement there are on Potentilleae two species under *Euphragmidium* and four species under *Earlea*. This places *Phragmidium Potentillae* (Pers.) P. Karst. under the section *Earlea*, a procedure not previously suggested. While uredinia occur regularly in the life-cycle, other characters, such as the non-hygroscopic pedicel, the smooth spore-wall and the absence of a well-defined apiculus on the teliospores, indicate that the affinities of this species are with the *Earlea* section. In the group on Rubeae four species are in the section *Euphragmidium* and one in *Earlea*, and of the eight species on Roseae only one is under *Earlea*.

### AECIAL CHARACTERS OF THE NORTH AMERICAN SPECIES

A critical study of the aeciospores shows that in these, also, there is a segregation into two distinct divisions based on the spore markings, divisions which do not, however, coincide with the sections *Earlea* and *Euphragmidium*. The predominant type of marking may be designated as verrucose with regular papillae. All of the species on Roseae and approximately half of those on Potentilleae and Rubeae have aeciospores of this type. *Phragmidium Rubi-idaei* (DC.) P. Karst. is so sharply papillose that it is more properly classed as strongly echinulate. The second type may be characterized as coarsely verrucose with irregular

warts and presents no gradation into the papillose type in any of the species studied. In the Roseae group it is necessary to take account of the spore wall thickness, the presence or absence of paraphyses, and the effect upon the host, all of which are features subject to some variation.

The determination of species by the examination of a single spore form is sometimes necessary but in general it is possible to collect and examine the other spore forms. When this can be done it will often be found that species with very similar teliospores have aeciospores of very contrasting character. This is especially true of *P. Rubi-idaei* and *P. Rubi-odorati*.

#### CHARACTERS OF THE FOUR SPECIES OF THE SECTION EARLEA ON POTENTILLEAE

In the section *Earlea* the following four species occur on Potentilleae: *P. Potentillae* (Pers.) P. Karst., on *Potentilla* species, *P. Ivesiae* Syd., on species of *Potentilla*, *Ivesia*, and *Horkelia*, *P. biloculare* Diet., on *Potentilla* species, and *P. Horkeliae* Garrett, on *Ivesia Gordonii* (Hook.) T. & G. The teliospores of *P. Potentillae* (PLATE 32, FIG. 9) are smooth and usually 4- to 5-celled and measure 23–30 × 48–89  $\mu$ , but in certain collections may be almost exclusively 3-celled, resembling in this respect *P. Ivesiae* (PLATE 32, FIG. 7) of which, however, the spores are smaller, measuring 19–26 × 35–51  $\mu$ , and the spore wall is verrucose at the apex. Both species have uredinia in the life-cycle. The teliospores of *P. Ivesiae* resemble in size those of *P. biloculare* (PLATE 32, FIG. 8), and *P. Horkeliae* (PLATE 32, FIG. 11), which, however, are 2-celled rather than 3-celled with a chestnut-brown rather than chocolate-brown spore wall. The teliospores of *P. biloculare* are coarsely verrucose with scattered tubercles and can readily be distinguished from the finely and sparsely verrucose spores of *P. Horkeliae*. Neither species produces uredinia.

Aecia are unknown in *P. Horkeliae*. The aeciospores of the other species are very similar, being rather strongly and evenly verrucose with regular papillae. The presence of paraphyses in the aecia of *P. Potentillae* distinguishes this species from *P. Ivesiae* and *P. biloculare* in which paraphyses are wanting. Aeciospore shape is not dependable in separating the latter two

species and it is doubtful whether satisfactory identification on morphologic grounds is possible without examining the teliospores.

#### CHARACTERS OF THE TWO SPECIES OF THE SECTION EUPHRAGMIDIUM ON POTENTILLEAE

The section *Euphragmidium* includes but two species on Potentilleae, *P. Andersoni* Shear, on *Dasiphora fruticosa* (L.) Rydb., and *P. Jonesii* Diet., on species of *Ivesia*, easily separable on either teliospore or aeciospore characters. *P. Andersoni* has 3- to 5-celled, very robust teliospores (PLATE 32, FIG. 10), which terminate in a hyaline papilla, or rarely in a distinct apiculus. In general the teliospore pedicels are one to one and one-half times the length of the spore, with the lower half swelling in water and becoming broadly lanceolate.

*P. Jonesii* has 5- to 9-celled, cylindric teliospores (PLATE 32, FIG. 1) which are somewhat narrowed below and bear at the apex a prominent apiculus. Characteristically the pedicel is shorter than the spore and swells abruptly to fan shape in the lower half, though it may vary, being in rare cases even attenuate.

The aeciospores of *P. Andersoni* are coarsely verrucose with irregular warts while those of *P. Jonesii* (PLATE 32, FIG. 1a) are so finely verrucose with papillae as to appear almost smooth when wet.

#### CHARACTERS OF THE ONE SPECIES OF THE SECTION EARLEA ON RUBEAE

On Rubeae the single representative of the section *Earlea* is *P. alaskanum* (Arth.) Sydow, known only on *Rubus stellatus* Smith, from southern Alaska. The 6- to 8-celled teliospores of this species are cylindric, with an apiculus, and a finely verrucose wall. As in most of the species on Rubeae the aeciospores are coarsely verrucose with irregular warts. There is no other species in North America likely to be confused with this one.

#### CHARACTERS OF THE FOUR SPECIES OF THE SECTION EUPHRAGMIDIUM ON RUBEAE

Four species on Rubeae are included in the section *Euphragmidium*: *P. Peckianum* Arth., on *Oreobatus* species; *P. Rubi-*

*idaei* (DC.) P. Karst., on *Rubus strigosus* Michx., and others; *P. Rubi-odorati* Diet., on *Rubus odoratus* L.; and *P. occidentale* Arth., on *Rubus parviflorus* Nutt. On the basis of teliospore characters alone the separation of certain of the species is difficult but *P. Peckianum* is distinct because the teliospores are only 4- to 6-celled, as contrasted with the 6- to 10-celled spores in the other three species, and are much more robust, tending to be ellipsoid rather than cylindric. The teliospores of *P. occidentale* are the most coarsely marked of any of this group, the markings appearing as prominent, hyaline beads. In relation to spore length the teliospore pedicels in *P. occidentale* are shorter than in *P. Rubi-idaei* or *P. Rubi-odorati*, being about equal to the spore length in *P. occidentale* and nearly one and one-half times the spore length in the other two species. In shape the teliospores of the three species last mentioned are much alike, although those of *P. occidentale* are somewhat narrowed below. The two species *P. Rubi-idaei* and *P. Rubi-odorati*, as will be shown later, are most readily distinguished by the use of aeciospore characters. However, the teliospores of *P. Rubi-idaei* (PLATE 32, FIG. 2) average  $26\text{--}30 \times 80\text{--}120 \mu$  and are somewhat longer and more slender than those of *P. Rubi-odorati*, which average  $29\text{--}34 \times 80\text{--}110 \mu$ .

With the exception of one species, the aeciospores of this group are of the type described as verrucose with irregular warts. The exception is *P. Rubi-idaei* the aeciospores of which (PLATE 32, FIG. 2a) are sparsely and strongly echinulate and once seen will be confused with those of no other species in North America. This species is, moreover, the only one in which the aecia are predominantly epiphyllous. Little can be said of the aeciospores of the other three species that is not embodied in the key which is presented later.

#### THE EIGHT SPECIES OF PHRAGMIDIUM ON ROSEAE

Following Dietel (4), Arthur (2) in 1909 published a critical review of the *Phragmidium* species on roses in North America in which he recognized six true members of the genus and in addition included a short discussion of *Phragmidium speciosum* (Fries) Cooke which he at that time considered as in the genus

*Earlea*. The total number of forms in these two genera, which were accorded specific rank, had reached nine at the time Arthur (3) in 1912 completed this section of the Uredinales for the North American Flora. In manuscript he has reduced this number to eight by considering *P. Rosae-setigerae* Diet. as a synonym of *P. americanum* (Peck) Diet., a combination which seems justified.

Morphological characters which can be pointed out as distinctive of any one species, as was possible in the species on Potentilleae, are scarce in the forms on Roseae. Certain species show marked tendencies in spore shape, size, color, or marking but intergradations are abundant and confusing. A definite knowledge of the host species is more essential here than in the other two groups.

#### CHARACTERS OF THE ONE SPECIES OF THE SECTION EARLEA ON ROSEAE

*P. speciosum* (Fries) Cooke, occurring on many wild and cultivated species of roses, is the sole representative of the section *Earlea* on roses and will, therefore, require little discussion. The teliospores are 4- to 8-celled, with a smooth ashy-brown wall. Typical infections can be recognized without a microscopic examination by the spindle shaped hypertrophy of the stems, bearing felty masses of spores. Aecia occur on leaves and stems, causing a marked distortion of the latter. The aeciospores (PLATE 32, FIG. 13) are thin walled, ellipsoid or more commonly oblong, and large, measuring 18–23 × 26–40  $\mu$ .

#### TELIOSPORE CHARACTERS OF THE SEVEN SPECIES OF THE SECTION EUPHRAGMIDIUM ON ROSEAE

The section *Euphragmidium* includes the following seven species on Roseae: *P. Rosae-arkansanae* Diet., on *Rosa arkansana* Porter, *R. Fendleri* Crépin, and *R. suffulta* Greene; *P. disciflorum* (Tode) J. F. James, on *Rosa gallica* L. and *R. alba* L.; *P. Rosae-pimpinellifolia* Diet., on *Rosa eglanteria* L., *R. hemisphaerica* Herrm., and others; *P. Rosae-acicularis* Liro, on *Rosa acicularis* Lindl., *R. nutkana* Presl, and others; *P. montivagum* Arth., on *Rosa Engelmanni* S. Wats., *R. Fendleri* Crépin, and others; *P. americanum* (Peck) Diet., on *Rosa carolina* L., *R. virginiana* Mill.,

*R. setigera* Michx., and others; *P. Rosae-californicae* Diet., on *Rosa californica* Cham. & Schlecht., *R. gymnocarpa* Nutt., *R. nutkana* Presl, and others.

For the species *P. Rosae-arkansanae* and *P. disciflorum*, both with 5- to 8-celled teliospores, the teliospore pedicel is, in typical cases, sufficiently characteristic to separate them from the other five species. The upper half of the pedicel is of uniform diameter and brownish tint. The lower half, however, when mounted in water swells abruptly to clavate or globose, and in *P. disciflorum* (PLATE 32, FIG. 3) characteristically disperses and disappears, often before a mount can be conveniently made and examined. Both have cylindric teliospores rounded at both ends, but those of *P. Rosae-arkansanae* (PLATE 32, FIG. 12) are more robust, measuring 26–29 × 62–80  $\mu$  as opposed to 22–30 × 64–90  $\mu$  in *P. disciflorum*, and the apiculus is very short. In the remaining species the teliospore pedicels swell gradually in the lower half to lanceolate or clavate.

*P. Rosae-pimpinellifoliae* (*P. subcorticium*), with 5- to 7-celled teliospores (PLATE 32, FIG. 4), is the only species on Roseae with spores which deviate strikingly from dark chocolate-brown. The teliospore wall is chestnut-brown and not especially opaque, a character so distinctive that no other need be mentioned. Aside from color the 5- to 9-celled teliospores of *P. montivagum* are similar to those of the preceding species; those of both species measure approximately 24–32 × 64–95  $\mu$  and are rounded above and below.

Of the three species which remain to be discussed *P. Rosae-acicularis* has the smallest and most finely verrucose teliospores (PLATE 32, FIG. 5). The spores are 5- to 11-celled, rather narrowly cylindric, and measure 19–29 × 48–100  $\mu$ . As in the teliospores of *P. Rosae-californicae* those of *P. Rosae-acicularis* tend to be spindle-shaped but the spores taper gradually, both above and below, and the limits of the apiculus can readily be made out. In *P. Rosae-californicae*, however, the spores (PLATE 32, FIG. 6) are strikingly acuminate above, with the apical cell usually much increased in length and grading directly into the apiculus, and the 8- to 11-celled teliospores also are considerably larger (24–32 × 80–130  $\mu$ ).

In *P. americanum* the teliospores are 8- to 11-celled, measure  $24-32 \times 64-125 \mu$ , and are cylindric, generally rounded or only slightly narrowed above. In size these approach those of the preceding species but the shape is distinctive and the pedicel is longer, averaging one and one-half times the spore length, while in *P. Rosae-californicae* the pedicel is about equivalent to the spore in length.

#### AECIAL CHARACTERS OF THE SEVEN SPECIES OF THE SECTION EUPHRAGMIDIUM ON ROSEAE

Segregation on the basis of aeciospore markings is not possible in this group, all species having spores of the regularly papillose type. The thickness of the spore wall, a less definite character, serves as a basis for a separation into two groups, one consisting of four species, *P. Rosae-californicae*, *P. Rosae-pimpinellifoliae*, *P. Rosae-acicularis*, and *P. montivagum*, in which the wall is  $2-3 \mu$  thick, and the other of three species, *P. disciflorum*, *P. Rosae-arkansanae*, and *P. americanum*, in which the wall is  $1-2 \mu$  thick.

Of the four species with thick aeciospore walls, *P. Rosae-californicae* typically has systemic mycelium, causes marked distortion of the host plant, and has aeciospores (PLATE 32, FIG. 6a) which are large ( $18-24 \times 27-40 \mu$ ). The aecia of *P. Rosae-pimpinellifoliae* are commonly on the stems but there is no noticeable hypertrophy, the spores (PLATE 32, FIG. 4a) are similar in size to those of the next two species, measuring  $13-19 \times 19-25 \mu$ , and paraphyses are few or none. The last character is sufficient to distinguish this species from *P. Rosae-acicularis* and *P. montivagum* which have abundant and conspicuous paraphyses. The separation of the latter two species is more difficult and depends mainly on aeciospore size, those of *P. Rosae-acicularis* (PLATE 32, FIG. 5a) being  $18-21 \times 24-30 \mu$ , those of *P. montivagum*  $16-19 \times 21-26 \mu$ .

In the group of three species with thin-walled aeciospores, *P. disciflorum* is characterized by sparsely and strongly verrucose aeciospores which are relatively large ( $18-24 \times 25-32 \mu$ ), and by the presence of abundant and conspicuous paraphyses. Paraphyses are present but usually few and inconspicuous in *P. Rosae-*

*arkansanae* and *P. americanum* and the spores measure 16–23 × 20–27  $\mu$ . The aeciospores of *P. Rosae-arkansanae* (PLATE 32, FIG. 12a) are strongly and sparsely verrucose or verrucose-echinulate, while those of *P. americanum* are more closely and finely marked.

#### EFFECT OF THE HOST SPECIES ON SPORE MORPHOLOGY

That the morphology of a species of rust is not unchangeable but is subject to some variation accordingly as it grows on one host species or another is well known and has previously been pointed out by Arthur (2) for certain species of *Phragmidium*, notably *P. montivagum*. The same general phenomenon has been noted in this study in two additional species. Collections of *P. Rosae-arkansanae* on *Rosa Fendleri* Crépin and of *P. Rosae-acicularis* on *R. nutkana* Presl yield teliospores of slightly greater diameter than those on other host species. No significant variation in length of teliospores is evident, the tendency being only to increase a few microns in width with a resulting spore of more robust appearance.

#### KEY TO THE SPECIES OF PHRAGMIDIUM BASED ON AECIAL CHARACTERS

The information derived from these studies of aecial characters in the North American species of *Phragmidium* has added somewhat to the data presented by Arthur (2), in 1909. The artificial key based on aecial characters which follows will perhaps be the most convenient and useful form in which to summarize the present knowledge of the *Phragmidium* species as discussed in this paper.

On the tribe Potentilleae.

Aecia unknown.

1. *P. Horkeliae*.

Aeciospores verrucose with irregular warts.

2. *P. Andersoni*.

Aeciospores verrucose with regular papillae.

Aeciospore-wall finely verrucose, appearing almost smooth.

3. *P. Jonesii*.

(Fig. 1a.)

Aeciospore-wall sparsely and strongly verrucose.

Paraphyses present and conspicuous. 4. *P. Potentillae*.

- Paraphyses absent.
- Aeciospores  $18-23 \times 23-26 \mu$ . 5. *P. Ivesiae*.  
(Fig. 7a.)
- Aeciospores  $16-21 \times 20-30 \mu$ . 6. *P. biloculare*.
- On the tribe Rubeae.
- Aeciospores very strongly echinulate. 7. *P. Rubi-idaei*.  
(Fig. 2a.)
- Aeciospores verrucose with irregular warts.
- Aeciospore-wall thin,  $1-1.5 \mu$ . 8. *P. alaskanum*.
- Aeciospore-wall thick,  $1.5-2.5 \mu$ . 9. *P. Rubi-odorati*.  
(Fig. 15.)
- Paraphysis-wall thickened above. 10. *P. occidentale*.
- Paraphysis-wall not thickened above.
- Aeciospores coarsely verrucose. 11. *P. Peckianum*.  
(Fig. 14.)
- Aeciospores moderately verrucose.
- Aeciospore-wall thin,  $1-2 \mu$ .
- Aeciospores large,  $18-24 \times 25-35 \mu$ .
- Aeciospores mostly oblong-ellipsoid. 12. *P. speciosum*.  
(Fig. 13.)
- Aeciospores mostly broadly ellipsoid. 13. *P. disciflorum*.  
(Fig. 3a.)
- Aeciospores small,  $16-23 \times 20-27 \mu$ .
- Aeciospores sparsely and strongly verrucose-echinulate. 14. *P. Rosae-arkansanae*.  
(Fig. 12a.)
- Aeciospores moderately and finely verrucose. 15. *P. americanum*.
- Aeciospore-wall thick,  $2-3 \mu$ .
- Infection not causing marked distortion of host.
- Paraphyses few or none. 16. *P. Rosae-pimpinellifoliae*.  
(Fig. 4a.)
- Paraphyses abundant and conspicuous.
- Aeciospores  $21-26 \mu$  long. 17. *P. montivagum*.
- Aeciospores  $24-30 \mu$  long. 18. *P. Rosae-acicularis*.  
(Fig. 5a.)
- Infection causing distortion; systemic. 19. *P. Rosae-californicae*.  
(Fig. 6a.)

**KEY TO THE SPECIES OF PHRAGMIDIUM BASED ON TELIOSPORE CHARACTERS**  
On the tribe Potentilleae.

Teliospore pedicels hygroscopic; § *Euphragmidium*.

Teliospores 3- to 5-celled.

1. *P. Andersoni*.  
(Fig. 10.)

- Teliospores 5- to 9-celled. 2. *P. Jonesii.*  
 (Fig. 1.)
- Teliospore pedicels non-hygroscopic; § *Earlea.*
- Teliospore-wall chocolate-brown.
- Teliospores smooth, 3- to 5-celled. 3. *P. Potentillae.*  
 (Fig. 9.)
- Teliospores verrucose at apex, 2- to 4-celled. 4. *P. Ivesiae.*  
 (Fig. 7.)
- Teliospores light chestnut-brown.
- Teliospores coarsely verrucose, 2-celled. 5. *P. biloculare.*  
 (Fig. 8.)
- Teliospores finely verrucose, 2-celled. 6. *P. Horkeliae.*  
 (Fig. 11.)
- On the tribe Rubeae.
- Teliospore pedicels hygroscopic; § *Euphragmidium.*
- Teliospores 4- to 6-celled. 7. *P. Peckianum.*
- Teliospores 6- to 10-celled.
- Teliospore pedicels once the spore length. 8. *P. occidentale.*
- Teliospore pedicels once and one-half the spore length.
- Teliospores  $26\text{--}30 \times 80\text{--}120 \mu$ . 9. *P. Rubi-idaei.*  
 (Fig. 2.)
- Teliospores  $29\text{--}34 \times 80\text{--}110 \mu$ . 10. *P. Rubi-odorati.*
- Teliospore pedicels non-hygroscopic; § *Earlea.* 11. *P. alaskanum.*
- On the tribe Roseae.
- Teliospore pedicels hygroscopic; § *Euphragmidium.*
- Teliospore pedicel swelling abruptly to broadly clavate or globose.
- Apiculus long,  $7\text{--}13 \mu$ . 12. *P. disciflorum.*  
 (Fig. 3.)
- Apiculus short,  $1\text{--}5 \mu$ . 13. *P. Rosae-arkansanae.*  
 (Fig. 12.)
- Teliospore pedicel swelling gradually to lanceolate.
- Teliospore-wall chestnut-brown. 14. *P. Rosae-pimpinellifoliae.*  
 (Fig. 4.)
- Teliospore-wall dark chocolate-brown.
- Teliospores rounded above.
- Teliospores  $64\text{--}95 \mu$  long. 15. *P. montivagum.*
- Teliospores  $64\text{--}125 \mu$  long. 16. *P. americanum.*
- Teliospores narrowed above.
- Teliospores  $48\text{--}93 \mu$  long. 17. *P. Rosae-acicularis.*  
 (Fig. 5.)
- Teliospores  $90\text{--}130 \mu$  long. 18. *P. Rosae-californicae.*  
 (Fig. 6.)
- Teliospore pedicel non-hygroscopic; § *Earlea.* 19. *P. speciosum.*

## SUMMARY

1. Segregation of the nineteen North American species of *Phragmidium* based on the character of the teliospore pedicel gives two sections, *Earlea*, including the six species whose pedicels are non-hygroscopic, and *Euphragmidium*, which includes the thirteen species with hygroscopic pedicels.

2. The species are arranged according to three tribes of the host-family Rosaceae, six species occurring on Potentilleae, five on Rubeae, and eight on Roseae.

3. The shape and size of the teliospores, the length and shape of the pedicels, the color and degree of marking of the wall, and the presence or absence of an apiculus are used as differential characters of the individual species within the sections *Earlea* and *Euphragmidium* on each of the three host tribes.

4. On the basis of aeciospore marking the species are discussed under two types as follows: five species have aeciospores which are verrucose with irregular warts and twelve species have aeciospores which are verrucose with regular papillae. In addition one species has strongly echinulate aeciospores, and in one species aecia are unknown.

5. The presence or absence of the aelial paraphyses and their characters are pointed out where characteristic of species.

6. The value of aeciospore size, shape, and wall thickness in the determination of species is discussed and the advantage of using both aeciospore and teliospore characters is emphasized.

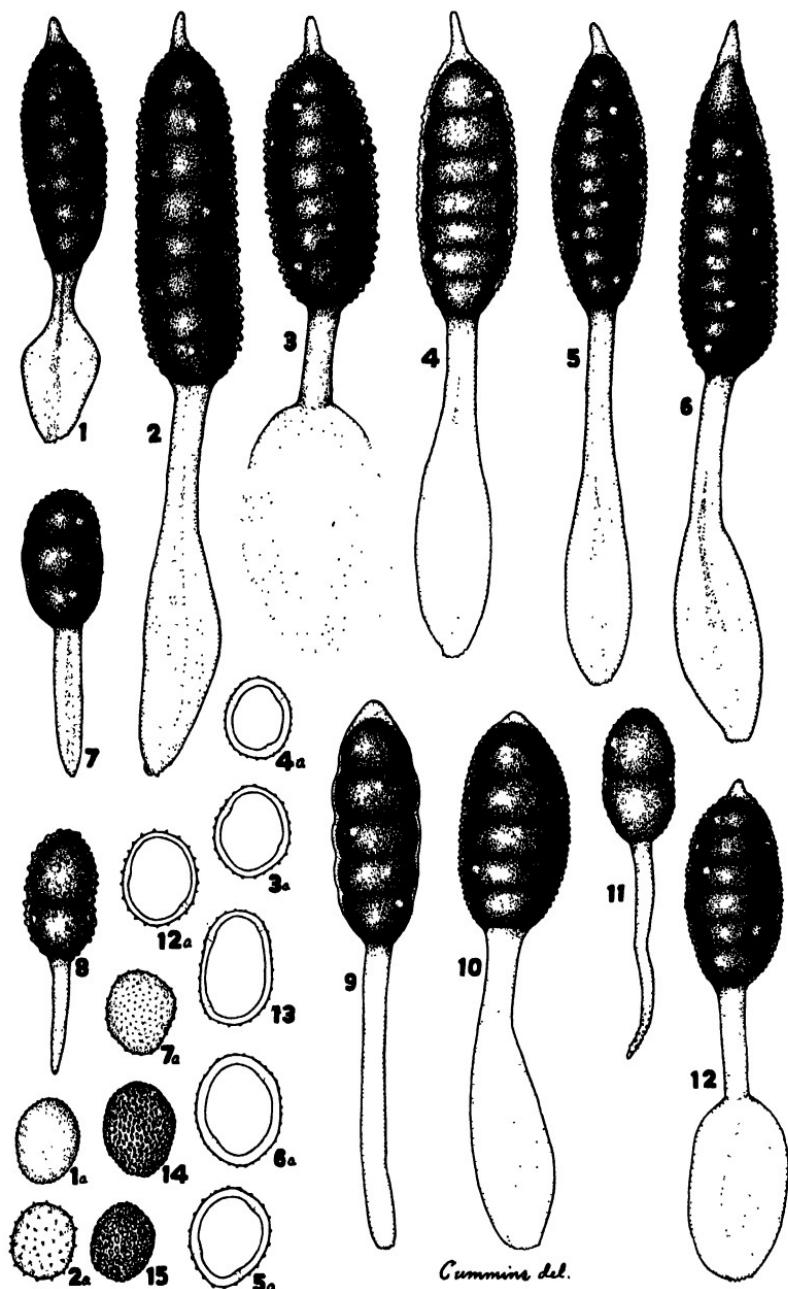
7. The effect of different host species on teliospore morphology is shown in certain species.

8. Keys to the North American species of *Phragmidium*, based on aelial characters and teliospore characters, are included.

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### EXPLANATION OF PLATE 32<sup>1</sup>

#### SPECIES OF PHRAGMIDIUM

Fig. 1, *P. Jonesii*, teliospore showing short fan-shape pedicel; 1a, *P. Jonesii*, aeciospore showing very finely verrucose wall; 2, *P. Rubi-idari*, showing large cylindric teliospore and long pedicel; 2a, *P. Rubi-idaei*, aeciospore showing strongly echinulate wall; 3, *P. disciflorum*, teliospore showing evanescent lower half of pedicel; 3a, *P. disciflorum*, optical section of an aeciospore showing thin wall; 4, *P. Rosae-pimpinellifoliae*, teliospore showing chestnut-brown wall; 4a, *P. Rosae-pimpinellifoliae*, optical section of an aeciospore showing thick wall; 5, *P. Rosae-acicularis*, showing spindle-shaped teliospore; 5a, *P. Rosae-acicularis*, optical section of an aeciospore showing thick wall; 6, *P. Rosae-californicae*, teliospore showing acuminate apex; 6a, *P. Rosae-californicae*, optical section of large thick-walled aeciospore; 7, *P. Ivesiae*, teliospore showing pedicel and verrucose spore apex; 7a, *P. Ivesiae*, aeciospore showing degrees of marking; 8, *P. biloculare*, teliospore showing evenly verrucose, chestnut-brown wall; 9, *P. Potentillae*, teliospore showing pedicel and smooth spore wall; 10, *P. Andersoni*, showing ellipsoid, non-apiculate teliospore; 11, *P. Horkelliae*, teliospore showing very finely-verrucose, chestnut-brown wall; 12, *P. Rosae-arkansanae*, teliospore showing short apiculus and broadly clavate pedicel; 12a, *P. Rosae-arkansanae*, optical section of an aeciospore showing thin, verrucose-echinulate wall; 13, *P. speciosum*, optical section of large, oblong aeciospore; 14, *P. Peckianum*, showing aeciospore moderately verrucose with irregular warts; 15, *P. Rubi-odorati*, showing aeciospore coarsely verrucose with irregular warts.

<sup>1</sup> All figures were made with the aid of a camera lucida and represent an enlargement of 420 diameters.

# A FURTHER STUDY OF THE MORPHOLOGY AND LIFE HISTORY OF THE ROSE BLACK SPOT FUNGUS

B. O. DODGE

(WITH PLATES 33-35)

One who attempts to grow hybrid roses in this part of the country is apt to find sooner or later some of them being defoliated by the black spot fungus, *Diplocarpon Rosae*. For example, at The New York Botanical Garden it has been observed for several years that certain varieties among our most beautiful roses such as Los Angeles, Mrs. Henry Bowles, Felicity and Padre, always show some spotting in spite of the fungicides which have been applied rather frequently. On the other hand, there are those roses like Red Radiance, Ethel Sommerset, Mrs. C. W. Edwards, La France, Radiance and others, which under the same treatment scarcely ever show black spot. The writer has been asked frequently why it is that the Red Radiance, for example, is comparatively resistant to this parasite. Is it because, as some say, the leaves probably have a cuticle which the fungus cannot penetrate? Or is it due to something more fundamental and inherent in the cytoplasm so that the fungus is unable to live even though it may readily penetrate the cuticle and so cause only a mere flecking. This is among the problems being studied, and some information on this question obtained as a result of preliminary experiments is also presented at this time.

## ARTIFICIAL INFECTION OF RED RADIANCE

Infection work was done in a greenhouse which is held in a rather dry condition. Although a number of potted roses of different varieties have been grown in this house for several years, black spot has seldom developed naturally. The amount of black spot, or the apparent resistance of a rose, evidently depends somewhat on the locality, and also on other conditions

which are apt to vary from year to year, so that a variety that does not happen to show spotting one year may be badly infected the next. Red Radiance was chosen to work with because of its general reputation as a fairly resistant variety. As a check or control Felicity was selected, as it has always shown black spot in our rose garden. The infection chamber employed is the familiar "iceless refrigerator" described by Hunt (4).

In order to learn whether the apparent resistance of Red Radiance is due primarily to the inability of the fungus to penetrate the cuticle of the host, inoculation of leaves was made by wounding them with a needle and then applying spore suspensions at the point of wounding. The leaves of the control Felicity plants were treated in the same manner. The inoculated plants were then left in cool damp chambers for at least two days and then removed to the bench. Ten days to two weeks later black spot began to appear on several leaves of both varieties at the points where they had been inoculated by wounding. This suggested, of course, that here was a type of resistance due to the nature of the cuticle and perhaps also of the cuticularized layers of the outer wall of the epidermal cell. It had been noted in the field that usually the brown or black discoloration of the leaf is, for some time at least, confined to the upper tissues, and in such cases the sori are to be found only on the upper side. Occasionally, however, one finds directly below the spot a small brown discoloration on the lower side showing a few small sori. This indicates that sometimes infection may occur naturally on the lower side of the leaf. After the fungus has gained entrance the mycelium could pass up through the mesophyll tissues and upper epidermis to spread out underneath the cuticle where sori would naturally develop. No one has described detailed experiments to prove that *Diplocarpon*, given an opportunity, does not sometimes gain entrance to the host through the stomatal openings which occur only in the lower epidermis. With this possibility in mind, areas on the lower epidermis of leaves of Felicity and Red Radiance were marked off with India ink and a drop of water containing spores was placed on each area. This was done with many leaves repeatedly and always with the same resulting high percentage of

infection in case of Red Radiance and Felicity, as well as other varieties which have since been tested. In no case, however, did sections of the area exposed to infection show hyphae penetrating the stomatal opening, although, as will be noted later, it very frequently happens that hyphal branches pass between the guard cells and their supporting cells.

While this work was being done, check experiments were made by placing drops of water containing spores on marked areas on the upper epidermis. In certain experiments the surface of the leaves was rubbed with the fingers so that a film of moisture would more readily adhere to the areas inoculated. Whenever fairly young leaves were chosen for the experiments, black spot developed on the Red Radiance just as frequently as it did on Felicity, proving conclusively that under the conditions maintained in these experiments Red Radiance can be artificially infected with black spot just about as easily as can Felicity, Padre, Mrs. Henry Bowles, Charles K. Douglas and other varieties tested. Rubbing the surface of the leaf is not at all necessary, as long as the drop of water containing the spores adheres to the leaf.

These experiments are, of course, far from a test of relative susceptibilities because the dosage in each case was large, and furthermore the number of individual penetrations per spot was not considered at all. Neither was the time during which the inoculated plants were kept in the damp chamber taken into account, except that it was planned to maintain conditions which would be most favorable for infection.

#### HOST-PARASITE RELATIONS

Wolf (7) has worked on the life history of this fungus and has shown that the black discoloration is due to the disorganization of the cells of the leaf and not to the mycelium of the fungus which is said to be practically colorless. He states that the fungus penetrates the cuticle of the upper epidermis, after which the hyphae run along in parallel strands, sending down here and there branches not only between the epidermal and palisade cells but also directly through them, so that the hyphae are intracellular as well as intercellular. As the sorus develops,

the host cells beneath become more or less disorganized, and the mycelium branches and develops abundantly in this region. Wolf's figures show hyphae penetrating the epidermal cells and also the palisade cells.

Our sections of infected leaves, some cut vertical to the leaf surface, others parallel to it (PLATE 33, FIGS. 5-7) show that, as stated by Wolf, the hyphae, soon after the spore germinates, grow in such a way that the branches run along parallel and close together, so that we soon have a layer of fungus tissue mostly one cell thick and made up of several parallel hyphae, which grow along directly beneath the cuticle.

Each hyphal cell has a single large nucleus with a distinct nucleole. Sections cut parallel to the surface of an infected leaf which have been fixed in Flemming's medium, stain readily so that the cytological details show beautifully. A terminal cell about to branch enlarges at the outer end where the nucleus comes to lie. In some cases observed two branches may form about the same time more or less dichotomously, although such a system of branching is not general here. Nuclear division occurs, each branch receiving one nucleus, after which walls cut off the branches from the parent cell. The two branches, at first widely diverging, immediately converge and become tightly pressed together and then continue growing on parallel, each hypha branching again independently. Some of the new branches join the others to broaden the fascicle, while others start off at broad oblique angles to develop a new subcuticular strand. One fascicle may cross another by dipping under it temporarily, coming back to the old level again after the passage is completed. While there is a general appearance of a hyphal system radiating from the points of infection (PLATE 33, FIG. 7), there are all sorts of criss-cross breaks so that a complex net-work is the final outcome (PLATE 34, FIG. 14).

#### HAUSTORIA

As for the subcuticular mycelium and the organization of the acervulus, the work of Wolf (7) has been confirmed. He says, however: "The internal mycelium penetrates the mesophyll of the leaf and furnishes nutriment for the subcuticular part. It

is connected with the latter by occasional hyphae which penetrate the epidermal cells or pass between them." Of the stroma of the acervulus he says: "It is connected with the internal mycelium below by hyphae which extend either through or between the epidermal cells into the mesophyll."

Sections of material fixed from two days up to two months, or even more in case of overwintered leaves, after inoculation, fail to show that mycelial hyphae actually pass through either the epidermal cells or the palisade cells below them. Figure 5 in plate 33 is from a photograph of a surface section at one focus, and figure 6 is of the same area at a slightly lower focus. At *A* in figure 5 are shown what look to be hyphae growing right through epidermal cells. These are in reality peculiar short hyphal branches growing across the top of these cells imbedded in the cuticularized layers of the outer walls. They are probably haustorium "mother cells." Their haustoria, as well as other haustoria, can be located in figure 6. Figures 7 and 8 are of the same general area but less highly magnified. It is clear from these pictures that we are dealing with typical haustoria and not with intracellular hyphae.

When one notes the very irregular outlines of epidermal cells with their re-entrant angles, it is not strange that one, viewing a section like that shown in figure 4 of plate 33, might be misled as to the nature of the hyphae which appear to be running right through the epidermal cell, when they are in reality intercellular. Figure 3, a photograph of the same section focused to take in the central plane, shows no internal hyphae. On the other hand, intercellular hyphae are very abundant (PLATE 34, FIG. 8), often completely enclosing an epidermal cell on all sides. In material three or four days old the haustoria are well developed in epidermal cells and numerous wherever hyphae are to be found. Figures 1 to 11 in plate 34 show various types of haustoria. Rather long narrow haustoria develop in palisade cells usually starting from the upper wall and extending straight down the cell. For example, three adjacent palisade cells (PLATE 34, FIG. 4) showed one such haustorium in each cell. They can scarcely be made out in this figure, however. Occasionally one finds a haustorium arising from a cell which appears to lie, if

not actually within the host cell cavity, at least not midway between two adjacent walls. Following such fungus cells by changing the focus usually proves that the hypha is, in fact, intercellular and only dips in deeper or splits the wall of the host cell about to be invaded by the haustorium.

The haustoria of *Diplocarpon* are of various forms and sizes, usually containing a single nucleus. The cytological details will be given in another paper. It may be said, however, that as compared with the haustoria of several species of the Asterineae described by Arnaud (1) and others, and with those of *Puccinia Sorghi* studied by Rice (5), there are no peculiarities that set them apart as wholly different. There is usually a bulge of the wall of the host cell at the point of penetration, a fine tube is sent through and a large cup-shaped stalk, probably composed of cellulose, is formed (PLATE 34, FIGS. 6, 7, 9, 10, 11). The haustorium proper penetrates this stalk increasing to its normal diameter just before it emerges. A distinct sheath is frequently visible (PLATE 34, FIGS. 4, 10). The nucleus is usually located at about the center of the haustorium (PLATE 34, FIG. 6). Some haustoria are long and hypha-like (PLATE 34, FIG. 1), while others are rather small showing little of the thickened cup-like base. A single host cell may contain six or eight haustoria, and occasionally one branches so that two or three seem to arise from the same mother-cell enlargement (PLATE 33, FIG. 5).

No evidence has been found to indicate any particular attraction or repulsion as to the relation of haustorium and host nucleus. Chloroplasts persist for some time in an invaded palisade cell.

Directly beneath an old acervulus there is some disorganization of the palisade, but in our material there is not the extensive breaking down of the palisade mesophyll noted by Wolf. Where leaves are allowed to winter over it is difficult to say whether the fungus cells one sees clearly within the epidermal cells are enlarged and perhaps dead haustoria, or whether hyphae may not actually penetrate dead host cells as saprophytic growth working decay. Later on in the spring when the fungus enters upon the development of the new structures about to be described, there is some crushing and destruction of old host cells which are replaced by fungus tissue.

When a leaf is infected through the lower epidermis the first hyphae run along under the cuticle, sending haustoria up into the epidermal cells, and hyphal branches push in between them. So loose is the spongy parenchyma in this region that the individual hyphae may occasionally be seen striking out across the open spaces. Where such hyphae do touch a cell there is apt to be a sort of appressorium developed which stains more deeply. No haustoria have been seen in cells of the spongy parenchyma, though they may no doubt be frequently present.

#### SPERMOGONIA

Higgins (3) in his very interesting studies of life histories of various ascomycetes has frequently noted the development of spermogonia so closely associated with infected areas as to indicate their genetic relationship to the species in question. No one has mentioned such structures in connection with the development of *Diplocarpon* on rose leaves. It may be of interest, therefore, to describe briefly fructifications which were frequently found during the month of March on the black spots of rose leaves which had been left out-doors over winter. Soon after these leaves are brought into the laboratory and placed in moist chambers small blister-like structures usually develop on some of the leaves at various points covered by the black spot fungus. Mounts will show that quantities of very small spermatium-like bodies are being formed from each of the cells of two-celled stalks. At first these stalks, because they were easily loosened from the points of their attachment, and because they resemble the conidia of *Diplocarpon*, were mistaken for ordinary conidia which were budding off microspores. The upper cell, however, instead of being the larger one, which is more often the case with the true conidia, is rather pointed or tapering (PLATE 34, FIG. 12). Furthermore, one would not infrequently find true conidia being developed in the same pustule, but independent of the growth of the microspores (PLATE 34, FIG. 13), in which case it was clear that we had true microconidia or spermatia as contrasted with normal two-celled conidia. No extensive germination studies have been made, but in the few attempts which were made to germinate the small bodies, no growth was seen during the week's

time they were closely observed. Under the same conditions the conidia germinated readily. Some of the pustules were wholly spermatial, others were wholly conidial, and still others were decidedly mixed.

Spermogonia or microconidia of *Phyllostictina* (*Phoma*) *carponema* are not infrequently found on rotting dewberries (2). Sections show that such structures may sometimes contain in addition to masses of spermatia, a few typical pycnospores. This should not be considered strange since spermogonia and pycnidia arise from the same gametophytic mycelium.

Stained sections of spermogonia on black spot of rose leaves show that the spermatia and the cells of their stalks are uninucleate, as are the cells of conidia. One finds in a young spermogonium a palisade layer of large spermatiophores thrusting the cuticle upward. Once space has been provided, spermatia first develop at the tip end of the stalk, and later on buds grow out from the lower cell at the septum. In old spermogonia the stalks are much reduced in size and one finds the spermatia formed as buds on mere knobs of growth. Whether one calls these little bodies spermatia or microconidia is immaterial until their function is ascertained. That they belong in the life history of *Diplocarpon* seems probable for the following reasons. They were found on infected leaves only on the black spots. They arise from a stroma of thick-walled dark-colored cells closely associated with the characteristic parallel hyphal filaments of *Diplocarpon*. They are like true acervuli in being subcuticular. The spermatiophores simulate the two-celled conidia of this fungus. True conidia which germinate readily are often found in unbroken spermogonia during the month of March. Cells of the spermogonial stroma, spermatiophores and spermatia are uninucleate. Furthermore, in view of the work of Higgins on various ascomycetes and of Wolf on *Diplocarpon*, and the facts presented below, the discovery of such additional fruiting structures of this fungus might well be expected.

#### ASCOCARPS AND SUBEPIDERMAL ACERVULI

Wolf (7) has given us a clear description of the development and morphology of the ascocarpic stage which he described as

the type of his new genus *Diplocarpon*. He believed that it must be mainly through this stage that the fungus lives through the winter because he was unable to find acervuli on infected old leaves in the spring. It has since become known, however, that the fungus frequently infects the canes and thus survives the winter and produces conidia the next season. Infection experiments carried out in the greenhouse during the past winter prove that one can usually infect roses by spraying them with water in which old infected rose leaves have been soaked for a few minutes. As noted previously, if these old leaves are brought into the laboratory in March and kept a few days in a moist chamber subcuticular acervuli may develop from the thick-walled dark-brown mycelial strands. These conidia germinate readily. The ascocarpic stage, then, in the climate of New York City is not essential for the spread of the fungus to the new spring growth.

At the close of the growing season of 1929 infected leaves were enclosed in a wire cage and then stored under shrubbery throughout the winter. Many of these old leaves were examined during the month of April but no ascocarps were found. The experiment was repeated in 1930. The leaves were carefully examined several times during March and April, 1931. If ascocarps were present they were not discovered. After each inspection the leaves were again exposed to the weather.

Wolf says that at Ithaca, N. Y., ascocarps begin to form sometime early in April and reach full maturity during that month. His developmental stages were obtained by fixing material at intervals during April. The circular perithecial shield is formed from radiating strands of thick-walled brown cells just beneath the cuticle. The fertile stroma develops between the epidermis and the palisade parenchyma. The central cells of the stroma are thin-walled and have stainable contents. It is from the basal portion of this tissue that paraphyses and asci are developed. The ascospores are two-celled and further resemble the conidia closely in color, size and shape, except that the ascospores are not so strongly constricted at the septum. They are discharged as the perithecium breaks open, and pile up in a whitish mass.

The production of ascocarps by a fungus is well known to be a matter of much uncertainty even in nature, depending on many factors not at all understood as yet. Evidently the difference between the cold winters of Ithaca with much snow, and the mild winters which have prevailed at New York City during the past few years could well account for the absence of ascocarps on our material. It is interesting, nevertheless, to find in our sections of infected spots on the old leaves fixed at intervals during March and April, structures (PLATE 35, FIGS. 4-7) which correspond very closely in their origin and mode of development with the ascocarps described by Wolf. The main difference being that in his structures paraphyses are formed and asci with ascospores are developed, while in ours the upward growing short filaments cut off conidia. Wolf's figures 6 to 10 of the early stages in the formation of the subepidermal ascocarps represent very well corresponding stages in the development of our internal fruit bodies. The latter have no definitely organized walls, still one might prefer to call them pycnidia. The same subcuticular crust of thick-walled cells covers the top like a shield in both cases. The radiating pattern of the shield elements is not striking however. The same massing of subepidermal stromatic tissue is found. The outer cells of the stroma are likewise thick-walled and carbonized, the inner ones are thin-walled and have stainable contents. Space is provided in the same way by crushing and raising the epidermis and thrusting aside the palisade tissue. The initial central filaments which develop within this stroma furthermore resemble the paraphyses with their thickened top ends figured by Wolf. They push up into the fungus tissue and remains of epidermal cells above. Here the exact duplication of structures in the various stages of development suffers a break in method or principle and yet the end products, ascospores and conidia respectively, are exactly alike and are discharged in similar whitish masses.

Authors have at various times described ascocarps which were said to have appropriated pycnidia or other asexual fructifications to their own use, asci, for example, appearing in the cavity, the basal sporogenous tissue of which had previously produced conidia. Such forehandedness has not been proved against

*Diplocarpon* as yet however. Only conidia have been found in the subepidermal fruit bodies on old leaves wintered over in the park at New York City.

The first conidia are cut off at the ends of rather thick filaments which are three or four cells long. A slight constriction occurs at the second septum and the upper portion is freed as a two-celled conidium. The remaining portion evidently can elongate and produce another spore, or become a spore in turn. Sections of a fruit body fixed after it had discharged a mass of spores (PLATE 35, FIG. 4) show very few filaments of the sort that initiate spore formation. The spores instead either have a one-celled stalk or arise directly from thin-walled sporogenous cells of the stroma. Crushed mounts of old material also show that two or three spores may arise at the same time from such cells and not have individual stalks as do the first spores to appear. The formation of a central cavity is mostly brought about through the upward thrust of these conidiophores, although some of the pressure is likely due to the partial disorganization and swelling, not only of a few of the thin-walled cells at the center, but also of some of the first formed spores. It enlarges just to the extent that the more deeply lying sporogenous cells take the place of those consumed in spore production.

The organization of the asexual fruit body of *Phoma (Phyllostictina) carpogena* (2) which is called a pycnidium is much the same. Why not call these deep-seated organs of *Diplocarpon* pycnidia instead of acervuli? The presence of a true ostiolar growth in one and not the other is immaterial. The *Phoma* has a more definite stroma the size of which is fixed before spore formation begins. In our old material the stroma above breaks away and exposes the sporogenous tissue (PLATE 35, FIG. 4) somewhat as is the case with the ordinary acervulus. Wolf refers to species of *Marssonnia* as having subepidermal acervuli. For the present at least we shall also refer to the deep-seated fruit bodies of *Diplocarpon* as subepidermal acervuli and to the normal summer type as subcuticular acervuli.

The conidia are discharged in a whitish mass as the acervulus breaks open. Wolf was unable to germinate the ascospores which he found heaped up in whitish masses, unless he placed

them in a drop of water on a rose leaf. The conidia formed in the subepidermal acervuli just described, on the contrary, germinate readily on an agar medium. Nevertheless we have here another striking parallelism not only in the method of the origin and development of the ascocarps and the subepidermal acervuli, but also in the color, form, size and type of germination of their ascospores and conidia respectively. This is plainly seen when one compares Wolf's figures of ascocarps and ascospores with our figures of the subepidermal acervuli and their conidia (PLATE 35). The strong resemblances between the ascocarps and the pycnidium of *Schizoparme straminea* (2) afford another beautiful illustration of such a parallelism.

That the subepidermal fruit bodies described above belong in the life history of *Diplocarpon* has been proved by inoculation experiments using cultures from single spores, so that there is no question in this regard. On the sixth day after inoculating the leaves, brown spots bearing typical subcuticular sori began to appear. At the end of the second week some eighty or more such spots had developed at points where drops of spore suspension had been placed. No spots developed on other leaves of these plants. The conidia which develop in these new sori are the same size and shape as are those shown in figure 1, plate 3, which were taken from a subepidermal fruit body. Conidia from cultures obtained from single spores from such sori are of this same type also. Regardless of the source of conidia they all germinate in the same characteristic fashion. The first germ tube is put out from a spot near the center of the upper, larger cell, and as it elongates, grows up into the air. The other cell then germinates and other germ tubes develop from the tip ends of both cells of the spore.

The conidia from an ordinary subcuticular acervulus shown in figure 2 seem to be more constricted at the septum. This may indicate a slight strain difference which can be followed out later by further inoculation experiments. Practically every one of the old leaves stored outside in two different cages developed subepidermal sori when they were finally brought into the laboratory during the last days in April. The main point brought out, in any event, is that when one inoculates a leaf with conidia

from a subepidermal sorus on over-wintered leaves, subcuticular sori are developed.

The tendency to form two-celled structures is manifested in four ways by this fungus. The spermatiophores are rather thick two-celled organs. The subcuticular acervuli develop two-celled hyaline conidia which resemble somewhat the spermatiophores. The subepidermal acervuli also form two-celled conidia which are even more like the two-celled ascospores described by Wolf than they are like conidia from ordinary subcuticular sori (PLATE 35, FIGS. 1 and 2). Furthermore the conidia in any old culture tend to develop very small two-celled secondary conidia.

Shear and Dodge (6) proved by many single spore cultures that *Pezizella Lythri* produces two genotypically different kinds of asexual fruit bodies. The sporodochial *Hainesia* stage develops exactly the same kind of spore as does the thick-walled sclerotium-like *Ptilidium* (*Sclerotiopsis*) pycnidium. The former is more adapted to spread the fungus widely during the summer. The latter stage often develops slowly during the winter and opens in the spring. The subcuticular acervuli of *Diplocarpon* are supplied with nourishment by a parasitic mycelium and provide conidia during the growing season of the host. The deep-seated subepidermal acervuli, on the other hand, are nourished in a purely saprophytic way by mycelium living on the dead host leaves, and provide spores for spring infection of the first rose leaves that appear.

It is encouraging to observe the great change coming about in the minds of botanists in recent years as regards sexual reproduction in the fungi. In view of the positive evidence being adduced that the spermatia of the rusts function in fertilization, there is a tendency to look with more favor on the suggestion of a similar function of the so-called microspores or spermatia of the ascomycetes. Proof that the structures which have developed on over-wintered rose leaves infected with *Diplocarpon*, and which have been called spermogonia in this paper, are connected with *Diplocarpon* is yet to be obtained. The fact that they frequently develop immediately above a subepidermal acervulus, and sometimes also form normal two-celled conidia, is evidence not to be disregarded. One can scarcely refrain from

hoping to find here another case of heterothallism, which would help to explain certain points regarding the formation of ascocarps as yet not clear.

Ascocarps, described by Wolf, but not seen in our material, develop on old leaves in April. They are deep-seated structures arising from a stroma between the epidermis and the palisade layer, and are capped by a shield of thick-walled brown cells which are developed in a radiating pattern from subcuticular hyphal strands above. Filamentous paraphyses with thickened tips first occupy the central cavity, then, by the end of April, asci with colorless two-celled spores appear. The ascospores are  $20-25 \times 5-6 \mu$ , not constricted at the septum. They are extruded in whitish masses as ascocarps break open. The ascospores germinate only in water on rose leaves.

The morphology of the structures described in this paper was worked out from crushed mounts and from stained slides. The writer is greatly indebted to Miss Marjorie Swift who made the stained preparations and who assisted in the experimental work. The cytological details and general discussion will be included in another paper.

#### SUMMARY

A description of the main features to be noted in a study of *Diplocarpon* on rose would include the following. Infection is most noticeable as black spots on the leaves, though the canes of certain varieties are subject to attack. Infection occurs directly through the cuticle on either side of the leaf. The superficial, primary mycelium is subcuticular and is composed of colorless uninucleate cells, the hyphae forming a network and tending to be associated in fascicles composed of several parallel filaments radiating from the point of infection. The internal mycelium is intercellular.

Haustoria, especially conspicuous in epidermal and palisade cells, are simple uninucleate structures, usually with a conspicuous thick cup-shaped stalk and a sheath sometimes fairly distinct. The black spot is due to disorganization of host cells and appears one or two weeks after inoculation.

Summer acervuli, usually on the upper side of the leaf, occa-

sionally on the lower, are subcuticular and contain two-celled colorless conidia arising from short inconspicuous cells of the thin basal stroma. The conidia,  $18-25 \times 5-6 \mu$ , are usually constricted at the septum and germinate readily in water or on agar media.

Spermogonia or microacervuli develop on the black spots on old leaves in March and April. They are subcuticular and usually on the upper side of the leaf. The spermatia are unicellular,  $2-3 \mu$  long, and resemble very small spores. They are cut off from two-celled stalks simulating the conidia except that they are smaller and taper upward. Sometimes normal two-celled conidia are present in spermogonia.

Internal deep-seated acervuli develop on old leaves early in April in a stroma located between the upper epidermis and the palisade layer. They are capped by a mass of thick-walled brown cells. They may rarely develop in the spongy parenchyma on the lower side of the leaf. The first conidiophores are filamentous and usually three or four cells long, the upper two cells being cut off as a true two-celled conidium. In old fruit bodies the conidia have a single stalk cell or arise directly from the sporogenous tissue cells, two or three spores often arising from the same cell, without having individual stalks. The conidia are colorless,  $20-25 \times 5-6 \mu$ , the upper cell usually thicker, not constricted noticeably at the septum. They are extruded in a whitish mass as the fruit body breaks open. The conidia germinate readily in water or on agar media.

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#### EXPLANATION OF PLATES

##### *Diplocarpon Rosae*

##### PLATE 33

Fig. 1. Section of old rose leaf showing subcuticular spermogonia March 22; Fig. 2. A deep-seated sorus with epidermal cells above much disorganized. Numerous two-celled spores loose in the cavity; Figs. 3, 4. Two views of the same section of a subcuticular acervulus at slightly different focuses to show that hyphae which appear to pass directly down through this epidermal cell are in reality running down between two adjacent cells; Fig. 5. Section of the upper epidermis cut parallel to the leaf surface. At A two haustorium mother-cells embedded in the upper wall of the epidermal cells; characteristic parallel strands of hyphae; Fig. 6. Same area at a slightly lower focus to show three or four haustoria in each cell directly beneath the fascicles of subcuticular surface hyphae shown in Fig. 5; Figs. 7, 8. Two views of the same general region as the preceding less highly magnified, fig. 8 at a lower focus. No haustoria in the light colored cells in the region where fig. 7 shows no subcuticular hyphae.

##### PLATE 34

Fig. 1. A large hypha-like haustorium in the cell at the left. A distinct sheath surrounds the haustorium in the cell at the right; Figs. 2, 3. Two views of the same host cells at different focuses. Cut ends of intercellular hyphae from which haustoria with large cup-shaped stalks arise are also visible. At the left in fig. 3 a cross section view of the cut end of haustorium stalk; Fig. 4. Epidermal cells beneath a young acervulus show sheathed haustoria. Each of three palisade cells also contains a haustorium running directly downward. Such a type might be mistaken for an intracellular hypha. They are not readily made out in this figure although distinct in the preparation; Fig. 5. A long epidermal cell showing eight or ten small haustoria with pouched ends; Figs. 6, 7 and 9. Other typical haustoria in epidermal cells; Fig. 8. From a section cut parallel to the leaf surface. Shows cut ends of many intercellular hyphae which surround these epidermal cells. The haustoria present in some of the cells do not show well in this picture; Fig. 10. The cellulose thickening extends well out around the haustorium; Fig. 11. A large haustorium growing down into a subepidermal cell of the type one sees above veins. The photograph was mounted sideways; Fig. 12. Shows the subcuticular spermogonial stroma of dark-colored hyphae and two-celled pointed spermatiophores; at the center a small two-celled conidium; Fig. 13. Section of a spermogonium which shows one of the several large two-celled conidia which were mixed in with the spermatia in this structure; Fig. 14. Subcuticular fascicles of hyphae crossing each other.

## PLATE 35

Fig. 1. Conidia from a subepidermal fruit body on an old leaf; Fig. 2. Conidia from a subcuticular acervulus of the normal summer type. The same magnification in figs. 1 and 2; Fig. 3. Spermatia massed in a typical subcuticular spermogonium; Fig. 4. Old subepidermal acervulus after discharging its white mass of conidia like those shown in fig. 1; Fig. 5. Young subepidermal fruit body just at the time the central cavity is beginning to be formed; Fig. 6. An older stage showing sporophores developing two-celled conidia. The two black areas above are old epidermal cells surrounded by thick-walled dark-colored fungus cells; Fig. 7. Fruit body nearly mature. Shows some conidia loose in the cavity. The stromatic tissue above is just breaking open. Figs. 5, 6 and 7 have the same magnification. Fig. 4 shows a still older stage and is less highly magnified.



DIPLOCARPON ROSAE





DIPLOCARPON ROSAE





DIPLOCARPON ROSAE



# THE RUSTS OF SOUTH AMERICA BASED ON THE HOLWAY COLLECTIONS—V<sup>1</sup>

H. S. JACKSON

## SPECIES ON EUPHORBIACEAE

168. AECIDIUM HYPSSOPHIILUM Speg. Anal. Mus. Nac. Buenos Aires 19: 321. 1909.  
*Euphorbia portulacoides* L. Termas de Chillón, Chile, Dec. 27, 1919, 251.

Holway's field notes indicate that this *Aecidium* is associated with *Uromyces andinus* P. Magn., but in his opinion it has no connection with it. Spegazzini considered this *Aecidium* to be connected with *U. hypsophilus* Speg. Tranzchel and Sydow have independently expressed the opinion that this *Uromyces* is the same as *U. andinus* and Sydow considers the connection doubtful. For these reasons I have listed the collection separately, with the possibility in mind that it is the aecial stage of a heteroecious species, the diplont of which occurs on Fabaceae. On the other hand, it would be interesting to have a study made of the germination of the spores of this *Aecidium*. It may well prove to be an *Endophyllum*.

### **Arthuria** Jackson, gen. nov.

Pycnia subcuticular, other sori subepidermal; aecia caeomoid, aeciospore wall echinulate; uredinia like the aecia, urediniospores catenulate with intercalary cells; telia semi-waxy, teliospores catenulate, thin-walled, germinating at once.

We name this genus in honor of Dr. J. C. Arthur, whose contributions to uredinology are well known. The structure suggests relationship with *Chrysomyxa* except that the aecia are

<sup>1</sup>Joint contribution from the Department of Botany, University of Toronto, and the Department of Botany, Purdue University Agricultural Experiment Station. Prepared with the aid of a grant from the American Association for the Advancement of Science. The fifth of a series of papers bearing the same title (see *Mycologia* 18: 139–163. 1926; 19: 51–65. 1927; 23: 96–116. 1931; 23: 332–364. 1931).

caeomoid and the pycnia subcuticular. It differs from *Bubakia* in the catenulate urediniospores.

169. *Arthuria catenulata* Jackson & Holway, sp. nov.

0. Pycnia chiefly epiphyllous, sometimes amphigenous, subcuticular, conspicuous, gregarious in small groups of three to eight, punctiform, flattened-hemisphaeric or occasionally conic, often confluent, 38–50  $\mu$  high by 75–100  $\mu$  broad.

I. Aecia caeomoid, chiefly hypophyllous, occasionally amphigenous, rounded or irregular, 0.3–0.8 mm. across, occurring singly opposite the pycnia or in groups, and then often confluent and circinating about the pycnia, early naked, pulverulent, golden or light cinnamon brown, ruptured epidermis conspicuous; aeciospores catenulate, obovate or ellipsoid, 18–25 by 26–38  $\mu$ ; wall colorless, 2–3  $\mu$  thick, sparsely and prominently echinulate, the pores obscure.

II. Uredinia hypophyllous, like the aecia in appearance and structure, scattered or gregarious, often with a large central sorus surrounded by an encircling group of smaller sori; urediniospores like the aeciospores.

III. Telia hypophyllous, scattered or gregarious, often with a large central sorus 0.6–1 mm. in diameter, surrounded by an encircling group of smaller sori, possibly developing in old uredinia, waxy or semi-waxy, at first golden-brown, becoming whitish on germination; teliospores catenulate, chains composed of 3–8 spores, slightly constricted at the septa, teliospores somewhat barrel shaped, 20–25 by 25–35  $\mu$ , adhering, germinating at once and in succession from apex to base of chain; wall thin, 1–1.5  $\mu$ , colorless, smooth; basidium stout, composed of four cells, basidiospores oblong, 11–13 by 18–20  $\mu$ .

*Croton celtidifolius* Baell. São Paulo, Brazil, Feb. 15, 1922, 1561.

*Croton* sp. Paineiras, Rio de Janeiro, Brazil, Aug. 17, 1921, 1046 (type); Petropolis, Rio de Janeiro, Brazil, Oct. 20, 1921, 1237; Bosque da Saude, São Paulo, Brazil, Jan. 31, 1922, 1518; Campos do Jordão, São Paulo, Brazil, Apr. 23, 1922, 1758.

I have little hesitancy in basing a genus on this interesting species. The combination of characters seems very different from anything previously described. It has not been possible to detect any essential difference between the aecia and uredinia or between the spores of the two stages. Should the uredinia be interpreted as repeating aecia?

It is possible that this may be the same as *Uredo Crotonis* P. Henn. Authentic material has not been available for comparison. In that species, however, the sorus is described as "ferrugino-ochraceis" and the urediniospores as having 4 equatorial pores. We have not been able to detect pores in our species.

The sori all appear to be naked, without peridia or paraphyses. Carefully made sections demonstrate beyond question that the spores in the aecia and uredinia are formed in the same manner, in chains with very evident intercalary cells.

**170. *Bubakia argentinensis* (Speg.) Jackson & Holway, comb. nov.**

*Uredo crotonicola* P. Henn. *Hedwigia* **35**: 251. 1896.

*Melampsora argentinensis* Speg. *Anal. Soc. Cient. Argent.* **47**: 266. 1899.

*Schroeteriaster argentinensis* Sydow, *Monog. Ured.* **3**: 401. 1914.

*Phakopsora argentinensis* Arth. *Bull. Torrey Club* **44**: 508. 1917.

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*Croton chaetophorus* Muell. Arg. São Paulo, Feb. 6, 1922, 1538.

*Croton hirtus* L'Hér. Bello Horizonte, Minas Geraes, Brazil, Dec. 4, 1921, 1365.

Two collections showing uredinia only have been included here, one of which is on the same host as the type of *Melampsora argentiensis*. The urediniospores are rather small, usually not exceeding 25  $\mu$  in length. The wall is rather closely and finely echinulate. The species seems to fit the revised uredinal description as given by Sydow (*Monog. Ured.* **4**: 578. 1924). The spores are occasionally slightly thickened at the apex, as noted by Sydow. *Uredo crotonicola* P. Henn. is included in the synonymy on the authority of Sydow, who has apparently compared authentic material. Arthur (*Mycologia* **14**: 13. 1922) assigned this *Uredo* to *Bubakia* (*Phakopsora*) *Crotonis* (Cooke) Arth. It seems best, however, to assign it to the above species for the present. It is still a possibility that the two species may be identical. More ample material of telia is needed before this question can be settled.

One of the collections listed above (1365) shows pycnia with primary uredinia. These are amphigenous, flattened hemisphaeric, 36–45  $\mu$  high by 65–100  $\mu$  broad, and occur with the primary uredinia on slightly hypertrophied spots. The urediniospores in the primary uredinia appear to be borne on short pedicels. If our identification is correct this is the first record of pycnia for a species of *Bubakia*, and indicates a brachy-type of life history.

171. ***Bubakia Ulei* (Syd.) Jackson & Holway, comb. nov.**

*Uredo Phyllanthi* P. Henn. *Hedwigia* 35: 249. 1896.

*Schroeteriaster Ulei* Sydow, *Ann. Myc.* 14: 70. 1916.

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*Phyllanthus brasiliensis* (Aubl.) Rusby. Coroico, Nor Yungas, Bolivia, June 11, 1920, 734.

The collection consists of uredinia only but seems to agree with the description given by Sydow, who assigns *Uredo Phyllanthi* P. Henn. as a synonym. Arthur's record of the latter from Trinidad (Thaxter 31) is an error. This specimen is the uredinal stage of a *Ravenelia*, probably *R. appendiculata* Lagerh. & Diet.

172. ***OLIVIA CAPITULIFORMIS* (P. Henn.) Arth. *Mycologia* 9: 61. 1917.**

*Uredo capituliformis* P. Henn. *Hedwigia* 34: 97. 1895.

*Ravenelia capituliformis* P. Henn. *Hedwigia* 43: 160. 1904.

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*Alchornea Iracurana* Casar. Rio de Janeiro, Brazil, Aug. 29, 1921, II, 1081.

*Alchornea pycnogyne* Muell. Arg. São Paulo, Brazil, Jan. 22, 1922, II, III, 1492.

A characteristic species which was originally described from Goyaz, Brazil. It is also known in the West Indies from Porto Rico and Tortala.

173. ***Puccinia Actinostemonis* Jackson & Holway, sp. nov.**

II. Uredinia amphigenous, scattered or gregarious, often on discolored spots, occasionally locally systemic and then occurring evenly distributed on stems, petioles and leaves, irregularly

circular in outline, 0.5–0.8 mm. across, often confluent, tardily naked, yellowish, pulverulent, ruptured epidermis noticeable; urediniospores broadly ellipsoid or ovoid, 16–18 by 18–23  $\mu$ ; wall colorless, 1.5–2  $\mu$  thick, evenly, closely and finely echinulate; pores obscure.

III. Telia amphigenous, chiefly hypophyllous, gregarious on discolored spots, often confluent, tardily naked, cinnamon brown, compact, ruptured epidermis conspicuous; teliospores clavate or cylindrical, 15–19 by 32–50  $\mu$ , rounded or obtuse above, rounded or narrowed below, slightly or not constricted, germinating at once with 4-celled basidium; wall light cinnamon brown, thin, 1–1.5  $\mu$ , thickened to 2.5–3  $\mu$  at apex, slightly thickened at angles near septum, smooth; pedicel short, colorless.

*Actinostemon* sp. Lapa, São Paulo, Brazil, Feb. 27, 1922,  
1600.

174. **Puccinia festata** Jackson & Holway, sp. nov.

? *Uredo Cornui* Har. Bull. Soc. Myc. Fr. 7: 147. 1891.

II. Uredinia chiefly hypophyllous, scattered, small, round, 0.2–0.5 mm. across, early naked, yellowish, compact, then pulverulent, ruptured epidermis not noticeable; paraphyses apparently chiefly peripheral, clavate or capitate, 60–75  $\mu$  long, the apex 18–25  $\mu$  broad, wall colorless or slightly tinted, uniformly thin, 1  $\mu$  or less; urediniospores broadly ellipsoid, 20–23 by 26–32  $\mu$ , wall colorless, thin, 1.5–2  $\mu$ , closely, evenly and minutely echinulate; the pores obscure.

III. Telia amphigenous, scattered, small, round, 0.2–0.8  $\mu$  across, tardily naked, pulverulent, blackish brown, ruptured epidermis conspicuous; teliospores irregularly ellipsoid, 25–32 by 38–56  $\mu$ , rounded below, rounded above with an abruptly acute umbo; wall chestnut brown, uniformly 2.5–3.5  $\mu$  thick, except at the umbo, evenly, closely, finely but prominently verrucose; pedicel colorless, tinted next to the spore, equalling the spore or shorter.

*Euphorbia*. Quito, Ecuador, Aug. 18, 1920, 912; Cuenca, Prov. del Aguay, Ecuador, Sept. 15, 1920, 990 (type); Huigra, Prov. Chimborazo, Ecuador, Aug. 6, 1920, 852.

The uredinal stage of this species agrees with a specimen collected by Lagerheim at Quito, Ecuador, December, 1899, which is assigned to *Uredo Cornui* Har. No authentic material of this species has been available, and the assignment of that *Uredo* to this species is problematical.

The second specimen listed (990), which is designated the type, consists primarily of uredinia with a few epiphyllous telia. The last collection listed (852) consists primarily of telia, these occur on either side of the leaf near the base. A very few uredinia occur on these leaves, leaving no doubt as to the identity of all the collections. The paraphyses which are abundant in the uredinia appear to be absent in the telia. These would bear a detailed study, as they seem to be borne in fascicles. Because of lack of time and the scanty material available no attempt has been made to work out the details of their formation.

175. RAVENELIA APPENDICULATA Lagerh. & Diet.; Dietel, Hedwigia 33: 47. 1894.

*Phyllanthus brasiliensis* (Aubl.) Rusby. Villa Aspiazu, Prov. Sur Yungas, Bolivia, May 31, 1920, 689.

Originally described from Ecuador, this species has otherwise been previously reported from South America only from Venezuela. It is also known in Central America from Guatemala and southern Mexico. Both uredinia and telia are present in the collection listed above.

176. *Uredo pavidæ* Jackson & Holway, sp. nov.

II. Uredinia hypophylloous, scattered, small, round, 0.2–0.4 mm. across, tardily naked, cinnamon brown, pulverulent, ruptured epidermis conspicuous; paraphyses clavate or cylindrical, scattered throughout the sorus, wall colorless, slightly thickened above; urediniospores broadly ellipsoid or obovoid, 16–20 by 26–34  $\mu$ ; wall golden brown, thin, 1–1.5  $\mu$ , in most spores uniformly thick, in a small percentage thickened at apex occasionally to 6  $\mu$ , moderately but sharply echinulate; the pores obscure.

*Croton compressus* Lam. Rio de Janeiro, Brazil, Aug. 29, 1921, 1080, Nov. 13, 1921, 1296; Campo Grande, Rio de Janeiro, Sept. 19, 1921, 1126; Campinas, São Paulo, Brazil, Apr. 3, 1922, 1694.

This *Uredo* is quite different from the one which we have assigned to *Bubakia argentiensis*. The spores are larger and more sharply and somewhat more sparsely echinulate. It does not seem to agree with the description of *Uredo Crotonis* P. Henn. In that species the soral characters are quite different,

the spores are larger, with thicker walls, the wall measurement is given as  $2\frac{1}{2}$ –4  $\mu$  by Sydow and no mention is made of paraphyses.

177. *Uredo Tijucae* Jackson & Holway, sp. nov.

II. Uredinia subcuticular, hypophylloous, on yellowish spots, scattered, small, round, 0.2–0.4 mm. across, early naked, cinnamon brown, pulverulent, ruptured cuticle conspicuous; urediniospores ellipsoid or obovoid, 15–18 by 24–25  $\mu$ ; wall thin, 1–1.5  $\mu$ , cinnamon brown with some tendency to be darker in upper part of spore, finely and closely verrucose; pores 2, equatorial.

*Phyllanthus lathyroides* H.B.K. Tijuca, Rio de Janeiro, Brazil, Aug. 19, 1921, 1056 (type); Petropolis, Rio de Janeiro, Brazil, Oct. 25, 1921, 1245.

It seems probable that this is the uredinial stage of a *Ravenelia*. It differs, however, from any described on this host genus. Paraphyses appear to be absent. Our specimens agree with Ule's collection 2214 from Rio de Janeiro issued as *Uredo Phyllanthi* P. Henn. It is quite different from that species, however, which has larger irregular spores and has been assigned by Sydow to *Schroeteriaster Ulei* Sydow.

178. *Uredo valentula* Jackson & Holway, sp. nov.

0. Pycnia subcuticular, amphigenous, gregarious in small compact groups, 0.3–0.8 mm. across, conspicuous, applanate, 38–64 by 88–115  $\mu$ .

II. Primary uredinia amphigenous, more commonly hypophylloous, in more or less circular groups, 0.5–1.5 mm., surrounding the pycnia, when infection involves a vein more elongate, to 5 mm. or more, cinnamon brown, early naked, pulverulent. ruptured epidermis noticeable; urediniospores obovate, broadly ellipsoid or occasionally pyriform, 15–18 by 25–31  $\mu$ ; wall colorless or slightly tinted, 1.5–2.5  $\mu$  thick, apex thickened, 3–5.5  $\mu$ , sparsely and rather strongly echinulate, pores 3, slightly super-equatorial.

II. Secondary uredinia few, scattered, small, round, 0.2–0.5 mm. across, chiefly epiphyllous, otherwise like the primary uredinia.

*Croton* sp. Therezopolis, Rio de Janeiro, Brazil, Oct. 15, 1921, 1221.

This primary *Uredo* suggests relationship with *Bubakia*. It appears amply distinct from all the other Holway collections

and does not answer to the description of *Uredo Crotonis* P. Henn. It is possible that it may be the primary stage of *Bubakia mexicana* Arth., but that species has not been recognized in South America. It would appear that there are more species of rusts on *Croton* than has been supposed. Their interrelationship cannot be settled till more material is available, especially of collections with teliospores.

179. **Uromyces Actinostemonis** Jackson & Holway, sp. nov.

II. Uredinia amphigenous, chiefly hypophyllous, gregarious in groups 0.5–1.5 mm. across on discolored spots, often confluent, occasionally locally systemic in actively growing tissues and then occurring evenly distributed on all parts, golden brown, small, irregularly circular, 0.2–0.3 mm. across, tardily naked, pulverulent, ruptured epidermis noticeable; urediniospores ellipsoid or obovoid, small, 12–14 by 15–19  $\mu$ , wall colorless, 1.5–2  $\mu$  thick, finely and closely echinulate, slightly more prominently so toward upper half of spore; pores obscure.

III. Teliospores in the uredinia, obovoid or oblong, 12–20 by 30–44  $\mu$ , rounded above, rounded or somewhat narrowed below; wall uniformly thin, 1  $\mu$  or less, colorless, smooth, germinating at once with no evidence of germ pore; pedicel colorless, short.

*Actinostemon concolor* Muell. Gavea, Rio de Janeiro, Brazil, Sept. 7, 1921, 1093; Bom Successo, Rio de Janeiro, Sept. 13, 1921, 1107; Copacabana, Rio de Janeiro, Brazil, Sept. 21, 1921, 1140.

180. **UROMYCES ANDINUS** P. Magn. Ber. Deutsch. Bot. Ges. 11: 48. 1893.

*Euphorbia portulacoides* L. Termas de Chillan, Chile, Dec. 27, 1919, 250.

This appears to be a short cycled form. The *Aecidium*, *A. hypsophilum* Speg. (see No. 168) often found associated with it is presumably the aecial stage of a heteroecious species with the diplont perhaps on Fabaceae. It is possible, however, that it may prove to be an *Endophyllum*. *U. hypsophilus* Speg. is probably a synonym.

181. **UROMYCES CISNEROANUS** Speg. Anal. Soc. Ci. Argent. 10: 134. 1880.

*Uredo Cisneroana* Speg. Anal. Soc. Ci. Argent. 17: 119. 1884.

*Sapium* sp. Therezopolis, Rio de Janeiro, Brazil, Oct. 15, 1921, III, 1219, II, 1223; Campos do Jordão, São Paulo, Brazil, Apr. 26, 1922, III, 1772; Reserva Florestal, Itatiaya, Brazil, May 14, 1922, III, 1854.

This characteristic species has previously been reported only from Argentina and Paraguay.

182. *UROMYCES PROËMINENS* (DC.) Pass. Rab. Fungi Eur. 1795. 1873.

*Uredo proëminens* DC. Fl. Fr. 2: 235. 1805.

*Trichobasis euphorbiaecola* Berk. & Curt. Jour. Linn. Soc. 10: 357. 1869.

*Uromyces Euphorbiae* Cooke & Peck; Peck, Ann. Rept. N. Y. State Mus. 25: 90. 1873.

*Uromyces euphorbiicola* Tranz. Ann. Myc. 8: 8. 1910.

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*Euphorbia heterophylla* L. Santa Clara above Lima, Peru, July 23, 1920, II, III, 789.

*Euphorbia hirta* L. Lapa, São Paulo, Brazil, March 3, 1922, I, 1608.

*Euphorbia hypericifolia* L. Riobamba, Ecuador, Aug. 11, 1920, I, 871.

*Euphorbia lasiocarpa* Klotsch. Santa Clara above Lima, Peru, July 23, 1920, II & III, 787; Cochabamba, Bolivia, Feb. 27, 1920, II, III, 340.

*Euphorbia thymifolia* L. Sorata, Bolivia, Apr. 11, 1920, II & III, 501; La Paz, Bolivia, Apr. 3, 1920, II, III, 494.

*Euphorbia* sp. Huigra, Chimborazo, Ecuador, Aug. 6, 1920, I, 854; Cochabamba, Bolivia, March 13, 1920, II, III, 405.

183. *Uromyces tolerandus* Jackson & Holway, sp. nov.

III. Telia hypophylloous, chestnut brown, scattered or gregarious in groups 3 mm. across, round, 0.5–1 mm., tardily naked, pulverulent, ruptured epidermis conspicuous; teliospores ellipsoid, 18–22 by 27–38  $\mu$ , rounded below, acute at apex; wall chestnut brown, 2–3  $\mu$  thick, acute apex thickened 5–8  $\mu$ , finely, evenly, and inconspicuously verrucose, pedicel colorless, equalling the spore or shorter.

*Manihot* sp. Reserva Florestal, São Paulo, Brazil, May 9, 1922, 1827.

No uredinia or urediniospores were found in this collection. The aspect, however, is not that of a micro-form. The species appears to differ sufficiently from any of the numerous species recorded on this and related hosts.

#### SPECIES ON ANACARDIACEAE

184. UREDO RHOMBICA Speg. Anal. Soc. Ci. Argent. 17: 124. 1884.

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*Astronium* sp. Rio de Janeiro, Brazil, Sept. 14, 1921, 1111; Jacarepagua, Rio de Janeiro, Brazil, Nov. 16, 1921, 1309.

A characteristic species easily identified by the rhomboid urediniospores. It appears to have been previously reported only from Paraguay.

#### SPECIES ON HIPPOCRATACEAE

185. Aecidium *Pereskiae* Jackson & Holway, sp. nov.

0. Pycnia epiphyllous, gregarious in a small group surrounded by aecia, prominent, deep seated, irregularly globoid or pyriform, 135–165  $\mu$  in diameter, ostiolar filaments present, not prominent.

I. Aecia epiphyllous, densely gregarious on somewhat hypertrophied discolored spots, surrounding the pycnia in a group 3–5 mm. in diameter, deep seated, bullate, small, 275  $\mu$  in diameter; peridium cupulate with margin erose or occasionally cylindrical, firm, white; peridial cells rhomboidal, 6–10 by 24–38  $\mu$ , outer wall 2.5–3  $\mu$  thick, smooth, inner wall 1.5–2.5  $\mu$ , prominently verrucose-tuberculate; aeciospores irregularly globoid or broadly ellipsoid, 25–38 by 25–40  $\mu$ ; wall thin, 1–1.5  $\mu$ , colorless, closely and finely verrucose.

*Pereskia grandifolia* Haw. Nictheroy, Rio de Janeiro, Brazil, Nov. 15, 1921, 1308 (type).

*Pereskia* sp. Friburgo, Rio de Janeiro, Brazil, Jan. 3, 1922, 1452.

Sections of this distinct species show a lining layer of mycelium to the aecidial cavity which persists after the aecia open and which accounts for the bullate appearance.

**186. *Uromyces Pereskiae* Jackson & Holway, sp. nov.**

II. Uredinia epiphyllous, gregarious on discolored spots 1.5–2.5 mm. across which appear greenish in dried specimens, round, 0.2–0.3 mm. in diameter, bullate, deep seated, cinnamon brown, tardily naked, becoming pulverulent, long covered by the firm overarched epidermis; urediniospores somewhat irregularly ellipsoid or obovate, 22–26 by 34–41  $\mu$ ; wall golden brown, 1.5–2.5  $\mu$  thick, sparsely and finely echinulate; pores 3 or 4 in a broad equatorial band.

III. Telia like the uredinia, blackish brown; teliospores somewhat irregularly broadly ellipsoid, 22–26 by 30–38  $\mu$ , rounded or truncate below, obtuse above; wall chestnut brown, 1.5–2  $\mu$  thick, appearing smooth but with irregularly scattered hyaline tubercles, sometimes arranged in lines and often more prominent at apex; pore prominent at apex but wall not thickened above; pedicel colorless, short, usually deciduous.

*Pereskia grandifolia* Haw. Fonseca, Nictheroy, Rio de Janeiro, Brazil, Sept. 18, 1921, 1123.

It is quite possible that this *Uromyces* is connected with the *Aecidium* described above as *Aecidium Pereskiae*. There is no evidence of the connection except that they are both collected in the same region and on the same host. It seems best, until more positive evidence is available of their identity, to describe the two forms as independent species.

**SPECIES ON SAPINDACEAE****187. PUCCINIA ARECHAVELATAE Speg. Anal. Soc. Ci. Argent. 12: 67. 1881.**

*Uromyces pervius* Speg. Anal. Soc. Ci. Argent. 17: 94. 1884.

*Uromyces aeruginosus* Speg. Revista Argent. Hist. Nat. 1: 20. 1891.

*Micropuccinia Arechavelatae* Arthur & Jackson, N. Am. Fl. 8: 541. 1922.

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*Cardiospermum Halicacabum* L. Huigra, Prov. Chimborazo, Ecuador, Aug. 3, 1920, 821; Pinheiros, São Paulo, Brazil, March 9, 1922, 1621.

*Serjania cuspidata* Camb. Bom. Successo, Rio de Janeiro, Brazil, Sept. 13, 1921, 1106; Gavea, Rio de Janeiro,

Brazil, Sept. 8, 1921, 1097; Fonseca, Nictheroy, Rio de Janeiro, Brazil, Sept. 18, 1921, 1121; Jacarépaguá, Rio de Janeiro, Brazil, Nov. 16, 1921, 1310.

*Serjania* sp. Santo Amaro, São Paulo, Brazil, Feb. 16, 1922, 1565; São Paulo, Brazil, Jan. 22, 1922, 1488; Juiz de Fora, Minas Geraes, Brazil, Dec. 17, 1921, 1408.

*Sapindaceae* (unidentified). Rio de Janeiro, Aug. 9, 1921, 1005; Aug. 10, 1921, 1010, Nov. 14, 1921, 1304; Nictheroy, Rio de Janeiro, Brazil, Aug. 18, 1921, 1052; Copacabana, Rio de Janeiro, Brazil, Sept. 21, 1921, 1141; Bello Horizonte, Minas Geraes, Brazil, Nov. 27, 1921, 1345, Dec. 1, 1921, 1352; Juiz de Fora, Minas Geraes, Brazil, Dec. 17, 1921, 1400, 1406; Campinas, São Paulo, Brazil, Apr. 3, 1922, 1695.

This characteristic microform has a wide distribution in South and Central America, as well as in the West Indies.

#### SPECIES ON RHAMNACEAE

188. *Puccinia Gouaniae* Holway, Ann. Myc. 3: 21. 1905.

*Uredo Gouaniae* Lagerh. Cf. Sydow, Monog. Ured. 4: 574. 1924.

*Bullaria Gouaniae* Arthur & Mains, N. Am. Fl. 7: 487. 1922.

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*Gouania polygama* (Jacq.) Urb. Therezopolis, Rio de Janeiro, Brazil, Oct. 1, 1921, 1179.

*Gouania corylifolia* Raddi. Campinas, São Paulo, Brazil, Apr. 4, 1922, 1698.

This and the following species have apparently not been previously recognized in South America except for a single collection from Colombia (Toro 293). The collections involved have been compared with authentic material and we have no hesitancy in assigning them to the North American species.

189. *Puccinia invaginata* Arth. & Johnston, Mem. Torrey Club 17: 146. 1918.

*Uredo Gouaniae* Ellis & Kelsey, Bull. Torrey Club 24: 209, 1897 (not *P. Gouaniae* Holway. 1905).

*Bullaria invaginata* Arth. & Mains, N. Am. Fl. 7: 488. 1922.

*Gouania* sp. Juquery, São Paulo, Brazil, June 12, 1922,  
1959.

190. PUCCINIA PARAENSIS Diet. Ann. Myc. 6: 96. 1908.

*Gouania Blancheana* Mig. Taquara, Rio de Janeiro, Bra-  
zil, Aug. 30, 1921, 1084.

A very characteristic brachy-form originally described on *Gouania pyrifolia* (?) from Marco, Para, Brazil. The primary uredinia occur on slightly hypertrophied spots often extending along the veins of the leaf. They are closely grouped, often confluent and occur on both sides of the leaf. Pycnia seem not to have been described but occur abundantly among the primary uredinia. They are amphigenous, inconspicuous, deep seated, globoid or nearly so, 120-140 by 140-160  $\mu$ , ostiolar filaments barely protruding.

The secondary uredinia and telia occur scattered on the under side of the leaves. The teliospore wall is often quite colorless, and the teliospores germinate as soon as mature.

#### SPECIES ON VITACEAE

191. ENDOPHYLLUM GUTTATUM (Kunze) Sydow, Ann. Myc. 17:  
179. 1920.

*Aecidium guttatum* Kunze in Weigelt exsicc. sine No. 1827.

*Aecidium circumscripum* Schw.; Berk. & Curt. Jour. Phil.  
Acad. Sci. II. 2: 283. 1853.

*Aecidium Cissi* Wint. Hedwigia 23: 168. 1884.

*Endophyllum circumscripum* Whetzel & Olive, Ann. Jour. Bot.  
4: 49. 1917.

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*Cissus sicyoides* L. Guayaquil, Ecuador, July 30, 1920, 795.

In assigning these collections to the *Endophyllum* the usual practice has been followed. In view of the probable origin of species of *Endophyllum* (see Jackson, H. S., Mem. Torrey Club 18: 1-108. 1931) it seems reasonable to expect that an unconnected *Aecidium* of a heteroecious rust, as will the *Endophyllum* derived from it, may both occur on this host.

192. PHAKOPSORA VITIS (Thüm.) Sydow, Hedwigia **38:** 141.  
1899.

*Uredo Vitis* Thüm. Pilze Weinst. 182. 1878.

*Physopella Vitis* Arth. Résult Sci. Cong. Bot. Vienne 338.  
1906.

*Vitis* sp. cult. Cuenca, Ecuador, Sept. 10, 1920, 975.

#### SPECIES ON TILIACEAE

193. *Didymopsora Triumfettae* Jackson & Holway, sp. nov.

0. Pycnia not seen, probably not formed.

III. Telia hypophyllous, occurring singly or in closely aggregated groups of 3–10 sori on slightly hypertrophied areas, groups usually more or less circular in outline, when associated with leaf veins more elongate; individual telia deep seated, waxy, forming cylindrical or terete columns to 1 mm. in height, 200–275  $\mu$  in diameter at base; peridium absent; teliospores catenate, two celled, without intercalary cells, 8–12 by 27–38  $\mu$ , slightly or not constricted at septum, two celled character not always evident; wall thin, colorless, apparently swelling considerably, germinating at once from apex to base of column.

*Triumfetta longicornis* St. Hil.? Juiz de Fora, Minas Geraes, Brazil, Dec. 17, 1921, 1405.

This very distinct species might easily be confused in the field with *Pucciniosira pallidula* (Speg.) Lag. The telia are more waxy in consistency and develop considerably longer than in that species, and are of considerably greater diameter at the base. Microscopically the two species are quite different. In this species there is no evidence of a peridium and a most careful search has failed to reveal the presence of intercalary cells, which are consistently present and quite easily demonstrated in the *Pucciniosira*.

I have assigned the species to *Didymopsora* as it seems to fit the characters of that genus better than any other. The two celled character of the spore is in some mounts very evident, in others one obtains the impression of chains of one celled spores suggesting *Endophylloides* or *Chionothrix*.

Elsewhere (Mem. Torrey Club **18:** 78–80. 1931) I have dis-

cussed the possible origin of *Pucciniosira* and similar genera and pointed out their resemblance to *Endophyllum*. In the species under discussion it seems possible to account for its origin from an *Endophyllum*-like species in which the spores have become vertically and laterally adherent due to the gelatinization of the cell wall, the peridium has been lost or has reverted to the ancestral condition and retained the spore function, and in which the intercalary cell cut off from the spore initial also becomes functional as a spore cell. It is noticeable that this species and the next as well as many other species in these and related genera have an *Aecidium*-like habit as shown by the deep seated character of the sorus initial and the tendency to be aggregated in close groups often on slightly hypertrophied areas.

194. PUCCINIOSIRA PALLIDULA (Speg.) Lagerh. Tröms. Mus. Aarsh. **16**: 122. 1894.

*Coleosporium* (?) *pallidulum* Speg. Anal. Soc. Ci. Argent. **17**: 95. 1884.

*Pucciniosira Triumfettae* Lagerh. Ber. Deuts. Bot. Ges. **9**: 344. 1891.

*Aecidium Triumfettae* P. Henn. Hedwigia **35**: 259. 1896.

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*Triumfetta semitriloba* L. Villa Aspiazu, Prov. Sur Yungas, Bolivia, May 31, 1920, 688; Friburgo, Brazil, Jan. 5, 1922, 1459.

*Triumfetta* sp. Guayaquil, Ecuador, July 31, 1920, 798; Jardim Bot., Rio de Janeiro, Brazil, Aug. 11, 1921, 1017.

A widely distributed species in South and Central America and the West Indies.

195. UREDO LUEHEAE Speg. Anal. Mus. Nac. Hist. Buenos Aires **23**: 31. 1912.

*Luehea* sp. Cantareira, São Paulo, Brazil, May 30, 1922, 1919.

This species seems to have been reported previously only from the type locality near Yuto, Juquy, Argentina.

## SPECIES ON MALVACEAE

196. CEROTELIUM DESMIUM (Berk. & Br.) Arth. N. Am. Fl. 7: 698. 1925.

*Aecidium desmum* Berk. & Br. Jour. Linn. Soc. 14: 95. 1875.

*Uredo Gossypii* Lag. Jour. Myc. 7: 48. 1891.

*Kuehneola Gossypii* Arth. N. Am. Fl. 7: 187. 1912.

*Kuehneola Gossypii* Butler, Fung. Dis. Pl. 363. 1918.

*Gossypium* sp. Nictheroy, Rio de Janeiro, Brazil, Nov. 15, 1921, 1307.

A well known species occurring throughout the world where cotton is grown.

197. CEROTELIUM MALVICOLUM (Speg.) Dietel in Engler, Nat. Pfl. II. 6: 57. 1928.

*Uredo malvicola* Speg. Anal. Soc. Ci. Argent. 17: 124. 1884.

*Rostrupia praelonga* Speg. Contr. Fl. Ventana 83. 1896.

*Kuehneola malvicola* Arth. N. Am. Fl. 7: 187. 1912.

*Pavonia sepium* St. Hill. Copacabana, Rio de Janeiro, Brazil, Sept. 21, 1921, 1139.

*Pavonia speciosa* H.B.K. Villa Augusta, São Paulo, Brazil, Feb. 25, 1922, 1598.

*Pavonia spinifex* Cav. São Paulo, Brazil, Jan. 19, 1922, 1480.

*Malvariscus* sp. (cult.). Rio de Janeiro, Brazil, Nov. 12, 1921, 1293, Dec. 20, 1921, 1410.

198. PUCCINIA INTERVENIENS (Peck) Bethel; Blasdale, Univ. Calif. Pub. Bot. 7: 119. 1919.

*Roestelia interveniens* Peck, Bull. Torrey Club 10: 74. 1883.

*Aecidium Malvastrum* P. Henn. Hedwigia 36: 216. 1897.

*Aecidium Sphaeralceae* Speg. Anal. Mus. Nac. Buenos Aires 19: 322. 1909.

*Allodus interveniens* Arthur & Orton, N. Am. Fl. 7: 797. 1927.

*Malvastrum capitatum* (Cav.) Griseb. Cochabamba, Bolivia, March 11, 1920, 397.

*Malvastrum* sp. La Falda, Cordoba, Argentina, Aug. 21, 1922, 2037.

*Sphaeralcea obtusifolia* Don. Zapallar, Chile, Sept. 22, 1919, 61.

*Sphaeralcea* sp. Viña del Mar, Chile, Sept. 10, 1919, 17.

These collections of aecidia were reported by Arthur (Proc. Am. Phil. Soc. **64**: 203. 1925) as the aecial stage of the heteropspis *Puccinia interveniens* which has telia on the grass genera *Nasella* and *Stipa*. The collections are reported here to make the record of malvaceous rusts in the Holway collections complete.

199. *PUCCINIA EXILIS* Sydow, Monog. Ured. 1: 481. 1903.

*Pavonia rosea* Schl. Lapa, São Paulo, Brazil, June 4, 1922, 1940.

This species seems to have been reported previously from South America only in connection with the original description. The type collection is from Brazil but no locality is given. The species is also known from Guatemala in Central America.

200. *PUCCINIA HETEROGENA* Lagerh. Jour. Myc. 7: 47. 1891.

*Althaea* sp. (cult.). Cuzco, Peru, June 29, 1920, 739.

*Malvaceae* (cult.). Quito, Ecuador, Aug. 26, 1920, 946; Biblian, Prov. de Cañar, Ecuador, Sept. 19, 1920, 965.

One of the above listed collections (946) is from the type locality for this distinct species which seems not to have been previously reported except from Ecuador.

201. *PUCCINIA HETEROSPORA* Berk. & Curt.; Berk. Jour. Linn. Soc. **10**: 356. 1868.

*Uromyces malvacearum* Speg. Anal. Soc. Ci. Argent. **12**: 72. 1881.

*Uromyces malvicola* Speg. Anal. Soc. Ci. Argent. **17**: 94. 1884.

*Micropuccinia heterospora* Arth. & Jackson, Arth. Bull. Torrey Club **48**: 41. 1921.

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*Sida cordifolia* L. Campinas, São Paulo, Brazil, Apr. 2, 1922, 1687; Santa Anna, São Paulo, Brazil, Feb. 22, 1922, 1589.

*Sida paniculata* L. Copacabana, Rio de Janeiro, Brazil,  
Sept. 21, 1921, 1132.

*Sida spinosa* L. Hacienda Anacuri, Prov. Nor Yungas,  
Bolivia, June 5, 1920, 725.

*Sida tomentosa* Mig. Friburgo, Rio de Janeiro, Jan. 6, 1922,  
1462.

*Sida urens* L. Hacienda La Florida, Prov. Sur Yungas,  
Bolivia, May 28, 1920, 674; São Caetano, São Paulo, Bra-  
zil, March 8, 1922, 1618; Prata, São Paulo, Brazil, Apr.  
7, 1922, 1705.

*Wissadula hernandioides* (L'Hér.) Garcke. Bello Horizonte,  
Minas Geraes, Brazil, Nov. 22, 1921, 1326.

202. PUCCINIA MALVACEARUM Bert. Mont. in Gay Fl. Chil. 8:  
43. 1852.

*Althaea* (cult.). Olmue, Chile, Sept. 11, 1919, 21.

*Malva* sp. Viña del Mar, Chile, Sept. 5, 1919, 5; San Felipe,  
Chile, Sept. 25, 1919, 66.

203. PUCCINIA PLATYSPORA (Speg.) Jackson & Holway, comb. nov.

*Uromyces platysporus* Speg. Anal. Mus. Nac. Buenos Aires  
6: 218. 1899.

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*Sphaeralcea* sp. La Falda, Argentina, Aug. 21, 1922, 2036.

The above specimen is from the region where this species has been reported. It seems to be distinct. A few two celled teliospores are present resembling somewhat the teliospores of *P. Sherardiana*. The great majority of the spores are one celled, however.

204. PUCCINIA SHERARDIANA Koern. Hedwigia 16: 19. 1877.

*Abutilon sylvaticum* (Cav.) Schum. Sorata, Bolivia, Apr. 19,  
1920, 555.

*Malva parviflora* L. Zapallar, Chile, Jan. 31, 1920, 305.

*Malvastrum coromandelianum* (L.) Garche. Rio de Janeiro,  
Brazil, Aug. 12, 1921, 1022; Juiz de Fora, Minas Geraes,  
Brazil, Dec. 17, 1921, 1409; Campinas, São Paulo, Brazil,  
Apr. 4, 1922, 1700.

*Makastrum* sp. Sorata, Bolivia, Apr. 14, 1920, 518.

*Sida rhombifolia* L. Cochabamba, Bolivia, Feb. 26, 1920,  
335.

#### SPECIES ON HYPERICACEAE

205. UROMYCES HYPERICI-FRONDOSI (Schw.) Arth. Bull. Minn. Acad. Nat. Sci. 2: 15. 1883.

*Aecidium Hyperici-frondosi* Schw. Schr. Nat. Ges. Leipzig 1: 68. 1822.

*Uromyces pachycephalus* Neger, Anal. Univ. Chile 93: 785. 1896.

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*Hypericum brasiliense* Choisy. Therezopolis, Rio de Janeiro, Brazil, Oct. 12, 1921, 1215; Petropolis, Rio de Janeiro, Brazil, Nov. 3, 1921, 1274; Alto da Serra, São Paulo, Brazil, Jan. 28, 1922, 1502.

*Hypericum chilense* Gay. Panimavida, Chile, Dec. 15, 1919, 235; Recinto, Chile, Jan. 9, 1920, 280.

*Hypericum* sp. Sorata, Bolivia, Apr. 19, 1920, 545; Ouro Preto, Minas Geraes, Brazil, Dec. 9, 1921, 1379; Friburgo, Rio de Janeiro, Jan. 5, 1922, 1460; Campos do Jordão, São Paulo, Brazil, Apr. 20, 1922, 1738.

#### SPECIES ON CLUSEACEAE

206. *Uredo Zarumae* Jackson & Holway, sp. nov.

II. Uredinia hypophylloous, closely gregarious in large areas 2–5 cm. across, evidently locally systemic, small, irregular, 0.2–0.4 mm. across, yellowish, early naked, pulverulent, ruptured epidermis conspicuous; urediniospores short stipitate, irregularly ellipsoid or oblong, 16–25 by 30–40  $\mu$ ; wall colorless, thin, 1–1.5  $\mu$ , closely, evenly and conspicuously verrucose.

*Clusia multiflora* H.B.K.? N. of Zaruma, Ecuador, Sept. 20, 1920, 996.

A very distinct species the sori of which occur closely gregarious in large irregular patches on the underside of the leaves evidently from an infection which becomes locally systemic. Sections indicate that the sori are intraepidermal and covered by the outer wall of the epidermal cells and the cuticle.

## SPECIES ON VIOLACEAE

207. PUCCINIA VIOLAE (Schum.) DC. Fl. Fr. 6: 62. 1815.  
*Aecidium Violae* Schum. Enum. Pl. Saell 2: 224. 1803.

*Viola maculata pubescens* Reiche. Temuco, Chile, Nov. 1, 1919, 152, Dec. 5, 1919, 197; Termas de Chillan, Chile, Dec. 31, 1919, 259.

*Viola* sp. Recinto, Chile, Jan. 10, 1920, 285.

## SPECIES ON LOASACEAE

208. UREDO FLORIDANA Sydow, Hedwigia 40: 129. 1901.  
*Blumenbachia lateritia* Hook f. Sorata, Bolivia, Apr. 17, 1920, 540.  
*Blumenbachia* sp. Huigra, Chimborazo, Ecuador, Aug. 3, 1920, 819.

This species has previously been reported only on the genus *Mentzelia* from Florida and Lower California in North America. The hosts are closely related and it seems best to record it as above as we can detect no essential difference between our collections and the North American material.

## SPECIES ON CACTACEAE

209. AECIDIUM OPUNTIAE P. Magn. Ber. Deut. Bot. Ges. 16: 151. 1898.  
*Opuntia sulphurea* Don. Cochabamba, Bolivia, March 1, 1920, 357.

Arthur, in his account of the South American grass rusts (Proc. Am. Phil. Soc. 64: 189. 1925), lists this aecidium as the aecial stage of *Puccinia Opuntiae* (Magn.) Arth. & Holway. The suggested connection of this aecidium with uredinia and telia on *Bouteloua simplex* Lag. is based on field observations only. While this surmise is doubtless correct, it seems best for the purposes of this account to list the collection as above.

## SPECIES ON LYTHRACEAE

210. Uredo cupheicola Jackson & Holway, sp. nov.

II. Uredinia hypophylloous, scattered or occasionally gregarious, round, 0.2–0.5 mm. across, cinnamon brown, tardily naked,

somewhat bullate, pulverulent, ruptured epidermis conspicuous; urediniospores irregularly ellipsoid or obovoid, 16–22 by 28–36  $\mu$ ; wall thin, 1– $1\frac{1}{2}$   $\mu$ , cinnamon brown, often lighter in color in lower half of spore, closely and finely verrucose; pores three approximately equatorial.

*Cuphea dipetala* (L. f.) Koehne. Sorata, Bolivia, Apr. 16, 1920, 531.

The sori of this clearly distinct species appear to have a somewhat indefinite pseudoperidium. The spore characters serve to separate it definitely from *Uredo Cupheae* P. Henn.

### 211. *Uredo Lafoenseae* Jackson & Holway, sp. nov.

II. Uredinia subepidermal, hypophylloous, scattered or gregarious, cinnamon brown, round, 0.2–0.4 mm., tardily naked, somewhat bullate, becoming pulverulent, ruptured epidermis conspicuous; urediniospores somewhat irregularly ellipsoid or obovoid, 16–19 by 22–31  $\mu$ , wall cinnamon brown, thin, 1  $\mu$  or less, moderately and finely echinulate, pores three, approximately equatorial.

*Lafoensis Pacari* St. Hil. Campos do Jordão, São Paulo, Brazil, Apr. 26, 1922, 1774.

This species differs from the preceding primarily in spore size, and from *Uredo Cupheae* P. Henn. in the somewhat larger spores and in the character of the wall markings. In *Uredo Cupheae* P. Henn. the markings are much more closely placed.

## SPECIES ON MYRTACEAE

### 212. *Puccinia Psidii* Winter, *Hedwigia* 23: 171. 1884.

*Caeoma Eugeniarum* Link, Sp. Plant 2: 29. 1825.

*Uredo neurophila* Speg. Anal. Soc. Ci. Argent. 17: 122. 1884.

*Uredo subneurophila* Speg. Anal. Soc. Ci. Argent. 17: 123. 1884.

*Uredo flavidula* Wint. *Hedwigia* 24: 260. 1885.

*Uredo Myrtacearum* Paz. *Hedwigia* 29: 159. 1890.

*Uredo Eugeniarum* P. Henn. *Hedwigia* 34: 337. 1895.

*Aecidium Glaziovii* P. Henn. *Hedwigia* 36: 216. 1897.

*Puccinia Jambosae* P. Henn. *Hedwigia* 41: 105. 1902.

*Uredo Goeldiana* P. Henn. *Hedwigia Beibl.* 42: 188. 1903.

*Uredo Myrciae* Mayor, Mem. Soc. Neuch. Sci. Nat. 5: 590. 1913.

*Puccinia Jambulana* Rangel, Bol. Agr. São Paulo **21**: 37. 1920.

*Puccinia subneurophila* Speg. Anal. Mus. Nac. Hist. Nat. Buenos Aires **31**: 32. 1922.

*Puccinia neurophila* Speg. Rev. Argent. Bot. **1**: 120. 1925.

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*Eugenia* sp. Nictheroy, Rio de Janeiro, Brazil, Nov. 15, 1921, 1306.

*Myrcia* sp. Campos do Jordão, São Paulo, Brazil, Apr. 26, 1922, 1775; Juquery, São Paulo, Brazil, Feb. 2, 1922, 1526.

*Psidium Guajava* L. Rio de Janeiro, Brazil, Nov. 14, 1921, 1302.

*Psidium* sp. Barbacena, Minas Geraes, Brazil, Dec. 12, 1921, 1383.

*Myrtaceae*, genus & sp. unknown. Bello Horizonte, Minas Geraes, Brazil, Nov. 26, 1921, 1342.

I have followed the combined treatment of Arthur (N. Am. Fl. **7**: 488. 1922), Sydow (Monog. Ured. **4**: 571. 1924), and Spegazzinii (Rev. Argent. Bot. **1**: 120, 127. 1925) in listing the synonyms of this much named species. Specimen No. 1775 on *Myrcia* bears teliospores; all the other collections are of uredinia; the teliospores on our specimen differ somewhat from the usual description, but we hesitate to erect a new name. They are irregularly ellipsoid or obovate, 18–20 by 32–48  $\mu$ . occasionally three celled and often with septa oblique, usually slightly constricted at septum and germinating at once; wall colorless, 1–1.5  $\mu$  thick, usually not thickened above but in occasional spores to 9  $\mu$ , smooth; pedicel colorless, equalling the spore, or more commonly shorter, often obliquely inserted.

#### 213. *Uredo seclusa* Jackson & Holway, sp. nov.

II. Uredinia hypophylloous, scattered, or more commonly gregarious on small purplish spots, small, irregularly circular in outline, cinnamon brown, tardily naked, pulverulent, ruptured epidermis conspicuous; urediniospores somewhat irregularly ellipsoid or obovoid, 15–20 by 24–32  $\mu$ ; wall thin, 1  $\mu$  or less, cinnamon brown, moderately and finely echinulate, the pores two or three, super-equatorial.

*Myrtaceae* sp. Villa Prudente, São Paulo, Brazil, May 31, 1922, 1925.

This *Uredo* is very distinct from the uredinia stage of *Puccinia Psidii* Wint. and appears to differ from any previously described.

#### SPECIES ON ONAGRACEAE

214. PUCCINIA EPILOBII-TETRAGONI (DC.) Wint. in Rab. Krypt. Fl. 1: 214. 1881.

*Uredo vagans Epilobii-tetragonii* DC. Fl. Fr. 2: 228. 1805.

?*Aecidium Oenotherae* Mont. in Gay, Hist. Chile 8: 37. 1852.

*Aecidium Gayophyti* Vize, Grevillea 7: 12. 1878.

*Puccinia Boisduvaliae* Peck, Bot. Gaz. 7: 45. 1882.

*Puccinia Gayophyti* Peck, Bot. Gaz. 7: 56. 1882.

*Puccinia Sphaerostigmatis* Dietel & Neger in Engl. Bot. Jahrb. 22: 353. 1896.

*Puccinia luxurians* Dietel & Neger in Engl. Bot. Jahrb. 24: 158. 1817.

*Puccinia Gayophyti* Speg. Anal. Mus. Nac. Buenos Aires III, 1: 63. 1902.

*Puccinia humilis* Speg. Rev. Arg. Bot. 1: 116-117. 1925.

*Boisduvalia concinna* Spach. Panimávida, Chile, Dec. 15, 1919, 230; Baños de Canquenes, Rancagua, Chile, Jan. 13, 1920, 298.

*Epilobium andicolum* Haussk. La Paz, Bolivia, March 24, 1920, 453.

*Godetia Cavanillesii* Spach. Papudo, Chile, Sept. 19, 1919, 50.

*Godetia* sp. San Jose de Maipo, Chile, Oct. 6, 1919, 91.

*Lavauxia mutica* Spach. Papudo, Chile, Sept. 18, 1919, 43; Panamavida, Chile, Dec. 9, 1919, 205.

*Lavauxia* sp. Viña del Mar, Chile, Sept. 7, 1919, 14.

*Oenothera mollissima* L. Panimávida, Chile, Dec. 13, 1919, 224.

*Oenothera* sp. Constitucion, Chile, Oct. 17, 1919, 121; Quito, Ecuador, Aug. 23, 1920, 939.

*Sphaerostigma tenuifolium* Spach. Papudo, Chile, Sept. 23, 1919, 65.

*Sphaerostigma* sp. Pichilemu, Chile, Oct. 12, 1919, 107.

I have followed the treatment of Bisby (Am. Jour. Bot. 3: 527-561. 1916) in assigning all these collections to the above species. As here considered, this species is apparently abundant in both North and South America.

#### SPECIES ON UMBELLIFERAE

215. AECIDIUM HYDROCOTYLINUM Speg. Anal. Mus. Nac. Buenos Aires 19: 321. 1909.

*Hydrocotyle Poeppigii* DC. Temuco, Chile, Nov. 1, 1919, 154, Dec. 7, 1919, 203.

*Hydrocotyle* sp. Valdivia, Chile, Nov. 13, 1919, 175.

A comparison of these collections with North American material assigned to *Uromyces Scirpi* shows that they are not the same. The type of *A. hydrocotylinum* Speg. has not been seen, but it seems best to record the above collections under that name. It is entirely possible that this is the *Aecidium* of *Puccinia Hydrocotyles*, but the life history of that species is unknown. The fact that *P. Hydrocotyles* has a wide distribution in both North and South America and has been frequently collected without its life history becoming certainly known would hardly support the view that an *Aecidium* is present in the life history.

216. PUCCINIA HYDROCOTYLES (Link) Cooke, Grevillea 9: 14. 1880.

*Caeoma Hydrocotyles* Link in Willd. Sp. Pl. 6: 22. 1825.

*Trichobasis Hydrocotyles* Cooke, Jour. Bot. 2: 343. 1864.

*Uredo Hydrocotyles* Bertero, Mont. Ann. Sci. Nat. II, 3: 356. 1835.

*Uredo bonariensis* Speg. Anal. Soc. Ci. Argent. 9: 171. 1880.

*Aecidiolum Hydrocotyles* Speg. Anal. Soc. Ci. Argent. 12: 80. 1881.

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*Hydrocotyle aconitifolia* Rich. San Felipe, Prov. Sur Yungas, Bolivia, May 19, 1920, 626.

*Hydrocotyle bonariensis* Lam. Arequipa, Peru, July 11, 1920, 772; Gavea, Rio de Janeiro, Brazil, Sept. 8, 1921, 1094.

*Hydrocotyle Bonplandii* Rich. Quito, Ecuador, Aug. 18, 1920, 899.

*Hydrocotyle umbellata* L. Huigra, Prov. Chimborazo, Ecuador, Aug. 6, 1920, 850.

*Hydrocotyle Volkmanni* Phil. Panamavida, Chile, Dec. 11, 1919, 219.

*Hydrocotyle* sp. Quito, Ecuador, Aug. 15, 1920, 897; Therezopolis, Rio de Janeiro, Brazil, Sept. 28, 1921, 1158.

217. PUCCINIA OBSCURATA Arth. & Holway; Arth. Am. Jour. Bot. 5: 477. 1918.

*Neonelsonia acuminata* (Benth.) C. & R. Quito, Ecuador, Aug. 18, 1920, 924.

This species is otherwise known only from the type locality in Guatemala.

218. UROMYCES MULINI Schroet. Hedwigia 35: 224. 1896.

*Uromyces Mulini* Speg. Anal. Mus. Nac. Buenos Aires 6: 218. 1899.

*Uredo mulinicola* Speg. Anal. Mus. Nac. Buenos Aires 6: 235. 1899.

*Uromyces Mulini* Schroet. var. *magellanica* Neger, Ofvers. Kongl. Vet.-Akad. Foch. 56: 746. 1899.

*Aecidium Azorellae* Speg. Anal. Mus. Nac. Buenos Aires 6: 230. 1899.

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*Mulinum spinosum* (Cav.) Pers. Termas de Chillan, Chile, Dec. 29, 1919, 255.

A characteristic species first reported from South America by Leveille in 1846 as *Uredo Cynapii* DC. The above collection includes both aecia and telia. The species is evidently an -opsis form.

219. **Puccinia discors** Jackson & Holway, sp. nov.

I. Pycnia amphigenous, gregarious in small groups, punctiform, deep seated, globoid or ellipsoid, 75–85 by 75–100  $\mu$ , ostiolar filaments not protruding.

II. Primary uredinia amphigenous, surrounding the pycnia; secondary uredinia amphigenous or petiolicolous, scattered or occasionally gregarious, round, often more elongate when on

veins or petioles, early naked, cinnamon brown, pulverulent, ruptured epidermis conspicuous; urediniospores obovate or ellipsoid, 25–28 by 32–38  $\mu$ ; wall cinnamon brown, 2.5–3  $\mu$ , considerably thickened, 5–7.5  $\mu$  at apex, moderately and finely verrucose echinulate; pores 3, approximately equatorial.

III. Telia like the uredinia, light chestnut brown, pulverulent; teliospores broadly ellipsoid or oblong, 23–28 by 35–44  $\mu$ , rounded above and below, scarcely constricted at septum; wall light chestnut brown, 2.5–3  $\mu$  thick, slightly thickened, 4–5  $\mu$ , over pore at apex of upper cell and over pore of lower cell, which is usually situated half way between septum and pedicel, evenly, closely and finely verrucose; pedicel colorless, short, deciduous.

*Conium maculatum* L. Papudo, Chile, Sept. 19, 1919, 51;  
Zapallar, Chile, Sept. 22, 1919, 62 (type), Jan. 31, 1920,  
303.

A distinct brachy-form. Sections show that teliospores may occur in primary uredinia.

## 220. *Puccinia mundula* Jackson & Holway, sp. nov.

0. Pycnia amphigenous, petiolicolous or caulicolous, honey yellow, punctiform, closely grouped among the aecia, deep seated.

I. Aecia amphigenous, petiolicolous or caulicolous, more commonly hypophyllous, in close groups on somewhat hypertrophied spots, groups elongate when involving veins petioles or stems, round, 0.4–0.8 mm. in diam., bullate, pulverulent; peridium present but soon breaking up into individual cells which occur scattered among the spores, peridial cells in face view 25–32  $\mu$ , wall prominently and closely verrucose; aeciospores somewhat irregularly globoid or ellipsoid, 15–20 by 20–30  $\mu$ ; wall colorless, thin, 1  $\mu$  or less, closely and finely verrucose.

II. Uredinia hypophyllous, scattered, round, 0.3–0.5 mm. across, early naked, golden brown, pulverulent, ruptured epidermis not noticeable; urediniospores ovoid or ellipsoid, 19–21 by 25–30  $\mu$ ; wall colorless or slightly tinted golden brown, 2.5  $\mu$  thick, finely and closely verrucose, pores three, approximately equatorial.

III. Telia like the uredinia, pulverulent, light chestnut brown; teliospores ellipsoid or oblong, 20–23 by 32–38  $\mu$ , rounded above, rounded or somewhat narrowed or truncate below, slightly or not at all constricted at the septum; wall light chestnut brown, 1.5–2  $\mu$  thick, slightly thickened, 3–4  $\mu$  at pores, with sparsely placed elongated thickenings often arranged in longitudinal lines; pedicel colorless, short, deciduous.

*Osmorrhiza* sp. Pongo, Prov. Sur Yungas, Bolivia, May 18, 1920, 610.

A very distinct species, quite unlike *P. Philippiae* Diet. & Neger. The aecia are of the sort often referred to as caeomoid. A peridium is present, however, as shown by sections. The peridial cells soon break apart and become scattered among the spores from which they are easily distinguished by their somewhat larger size and coarser wall markings. The markings of the teliospores are peculiar and easily overlooked, the effect is to give the margin of the spore a somewhat wavy appearance. The true nature of the thickenings is best seen in lacto-phenol.

221. *Puccinia Philippiae* Dietel & Neger, Engl. Bot. Jahrb. 22: 352. 1896.

*Aecidium Philippiae* Speg. Bol. Acad. Nac. Ci. Cordoba 27: 355. 1924.

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*Osmorrhiza Berteri* DC. Concepcion, Chile, Oct. 26, 1919, 139; Peulla, Lago Todos los Santos, Chile, Nov. 30, 1919, 194.

*Osmorrhiza* sp. Temuco, Chile, Nov. 1, 1919, 153; Pani-mávida, Chile, Dec. 15, 1919, 232.

The first two specimens listed are on the same host as the type collection and from the same region. The species has also been collected in Argentina and Patagonia.

222. *Puccinia repentina* Jackson & Holway, sp. nov.

II. Uredinia not seen; urediniospores in the telia obovate, 21–25 by 30–37  $\mu$ ; wall golden brown, 2–2.5  $\mu$  thick, thickened at apex, 7–10  $\mu$ , and frequently also at base, moderately and finely echinulate, pores somewhat obscure, apparently three, approximately equatorial.

III. Telia hypophylloous, round, 0.3–0.5 mm. across, blackish brown, early naked, pulverulent, ruptured epidermis not conspicuous; teliospores somewhat irregularly ellipsoid or oblong, 23–28 by 32–45  $\mu$ , rounded above and below, scarcely constricted; wall chestnut brown, 2.5–3.5  $\mu$  thick, thickened slightly over pores, 4–6  $\mu$ , very finely and closely verrucose; pedicel colorless, short, deciduous.

*Arracacia xanthorrhiza* Bauer. Sorata, Bolivia, Apr. 22, 1920.  
563.

This species is quite different from others previously described on this host genus and does not appear to be the same as any reported on closely related hosts. The pore in the lower cell of the teliospore is usually close to the septum, sometimes slightly depressed. The wall markings are so fine that the spores appear nearly smooth in water mounts.

#### SPECIES ON CORNACEAE

223. PUCCINIA GRISELINIAE Pazschke, Hedwigia 35: 52. 1896.

*Griselinia ruscifolia* Taub. Serra Itatiaya, São Paulo, Brazil, May 17, 1922, 1855.

The collection of this interesting micro-form was apparently made near the type locality. It is otherwise known only from the original collection (Ule, 2101) and one other (Ule, 1642) made at Serra Geral, Brazil.

#### SPECIES ON ERICACEAE

224. UREDO ANDROMEDAE Cooke, DeToni, in Sacc. Syll. Fung. 7: 853. 1888.

*Pernettya Pentlandii* DC. Sorata, Bolivia, May 7, 1920, 594.

This form is not unlike several North American collections on *Pieris* and *Xolisma*, which were originally named *Uredo Andromedae* by Cooke and are now assigned by Arthur, in the North American Flora Supplement, to *Pucciniastrum Myrtilli* (Schum.) Arth. There seems to be some reasonable basis for doubt that they belong with the latter species. Our assignment of the collection listed above to *Uredo Andromedae* is merely tentative.

#### SPECIES ON MYRSINACEAE

225. UROMYCES MYRSINES Diet. Hedwigia 36: 26. 1897.

*Uromyces Rhapaneae* P. Henn. Hedwigia 48: 1. 1908.

*Uromyces Usterianus* Diet. Ann. Myc. 6: 96. 1908.

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*Icacorea* sp.? Poços da Caldas, São Paulo, Brazil, Apr. 10, 1922, 1721.

*Rapanea pseudocrenata* Maz.? Hacienda La Florida, Prov. Sur Yungas, Bolivia, May 26, 1920, 662; Villa Aspiazu, Prov. Sur Yungas, Bolivia, June 1, 1920, 695; Coroico, Prov. Nor Yungas, Bolivia, June 11, 1920, 730.

*Rapanea umbellata* (Mart.) Mez. Therezopolis, Rio de Janeiro, Brazil, Oct. 26, 1921, 1248.

*Rapanea* sp. Campos do Jordão, São Paulo, Brazil, Apr. 28, 1922, 1782.

This characteristic short cycle species has previously been reported only from Brazil. According to Sydow *U. uestrianus*, originally described as on a member of the Myrtaceae, is really on Myrsine.

#### SPECIES ON GENTIANACEAE

##### 226. *Puccinia Lisianthi* Jackson & Holway, sp. nov.

II. Uredinia hypophyllous and caulicolous, gregarious, large, 0.5–1 mm., round or oval in outline, cinnamon brown, deep seated, tardily naked, pulverulent, long covered by the over-arching epidermal and subepidermal tissues; urediniospores obovoid or ellipsoid, 18–21 by 25–34  $\mu$ ; wall colorless or slightly tinted golden brown, 1–2  $\mu$  thick, rather closely and finely echinulate with smooth areas surrounding the pores; pores two, equatorial or slightly superequatorial.

III. Telia like the uredinia, compact, cinnamon brown; teliospores cylindrical or elongate terete, 14–18 by 44–56  $\mu$ , obtuse above, narrowed and truncate or less commonly rounded below, constricted at septum; wall colorless or slightly tinted golden brown, 1.5  $\mu$  thick, apex thickened 3–4  $\mu$ , smooth; pedicel colorless, equaling the spore or longer, broad at point of attachment, 5–6.5  $\mu$ , soon collapsing.

*Lisianthus elegans pedunculatus* Cham. & Schl. Ouro Preto, Minas Geraes, Brazil, Dec. 8, 1921, 1372.

An apparently distinct form in which the uredinia appear to be locally systemic. The uredinia are very characteristic, originating quite deeply in the tissues and covered by the epidermis and three or four layers of the rather compact spongy parenchyma. The hymenium of the sorus is flat as in those forms in which the sorus originates just beneath the epidermis. Teliospores are not abundant in this material but occasionally occur in sori essentially like the uredinia and germinate at once.

## SPECIES ON APOCYNACEAE

227. AECIDIUM OCHRACEUM Speg. Rev. Argent. Hist. Nac. Buenos Aires 1: 401. 1891.

*Tabernaemontana amygdalifolia* Jacq. Friburgo, Rio de Janeiro, Brazil, Jan. 5, 1922, 1458.

It is probable that the record from Paraguay of *A. ceraceum* Berk. & Br., made by P. Hennings (Hedwigia 35: 257. 1896), is an error for the above species. *A. ceraceum* is, according to Sydow (Monog. Ured. 4: 320. 1923), the aecial stage of *Puccinia Tabernaemontanae* Berk. & Br., which is known only from Ceylon, Natal and Zululand. The aeciospores of *A. ochraceum* Speg. are quite different, and it is probable that it is heteroecious.

228. **Puccinia Mandevillae** Jackson & Holway, sp. nov.

0. Pycnia not seen, probably not formed.

III. Telia hypophylloous, gregarious in small groups 1-4 mm. across, irregularly rounded, commonly confluent, early naked, compact, becoming pulvinate and cinereous through germination, cinnamon brown, ruptured epidermis at first noticeable; teliospores ellipsoid, clavate or oblong, 14-18 by 29-38  $\mu$ , rounded above, rounded or narrowed to pedicel below, slightly or not constricted at septum, which is ordinarily transverse, occasionally oblique; wall colorless or slightly tinted golden brown, 1-1.5  $\mu$  thick, thickened to 3  $\mu$  at apex, smooth; pedicel colorless, once to twice the length of the spore, 6-7  $\mu$  broad at point of attachment, soon collapsing.

*Mandevilla Mandoni* Rusby. Sorata, Bolivia, Apr. 19, 1920, 556.

While somewhat like *Puccinia obliqua* Berk. & Curt., which occurs on members of the Asclepiadaceae, this micro-form seems sufficiently distinct morphologically, and occurs on a different host family. The septum is only rarely oblique.

It is especially interesting to note that the teliospores germinate with a two celled basidium. Elsewhere (Mem. Torrey Club 18: 22-27. 1931) I have reviewed the available knowledge with reference to the occurrence of two celled basidia in micro- and endo-forms, and have discussed the probable significance of this phenomenon. The two celled basidium provides an easily determined external indication of a simplified nuclear history. Spe-

cies in which the spores germinate in this way should be well worth investigating cytologically.

229. *Uredo Condyllocarpi* Jackson & Holway, sp. nov.

II. Uredinia hypophyllous, scattered or somewhat gregarious, small, round, 0.2–0.3 mm. across, cinnamon brown, early naked, pulverulent, ruptured epidermis not conspicuous; paraphyses abundant, peripheral, at first incurved, becoming somewhat erect, 60–225  $\mu$  in length, 6  $\mu$  wide at apex, up to 12  $\mu$  at base, wall thin, colorless or light golden brown, 1–1.5  $\mu$ , sometimes thickened to 4  $\mu$  on outer side at base; urediniospores ellipsoid, obovate or pyriform, 19–25 by 32–38  $\mu$ ; wall thin, 1–1.5  $\mu$ , light cinnamon brown, closely and minutely echinulate, the pores obscure.

*Condyllocarpon Rauwolfiae* Muell. Arg. São João, São Paulo, Brazil, July 2, 1922, 1986.

The very long erect paraphyses make this a well marked species. It is possible that it may be the same as *Uredo Apocynaceae* P. Henn., which was described from Brazil on an unknown member of the Apocynaceae. Authentic material has not been available. In that species the paraphyses are described as being only 35–65  $\mu$  long.

SPECIES ON ASCLEPIADACEAE

230. PUCCINIA ARAUJAE Lév. Ann. Sci. Nat. III. 5: 69. 1845.

*Puccinia Schnyderi* Speg. Anal. Soc. Cient. Arg. 10: 8. 1880.

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*Arauja sericifera* Brot. Pinheiros, São Paulo, Brazil, March 9, 1922, 1620.

A short cycle form apparently sufficiently distinct to justify recognition. The spores are more elongated than in *P. obliqua*.

231. PUCCINIA CONCRESCENS Ellis & Ev.; Arth. Mycologia 7: 240. 1915.

*Puccinia compacta* Kunze in Sydow, Monog. Ured. 1: 334. 1902 (not Berk. 1855).

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*Asclepias curassavica* L. Juiz de Fora, Minas Geraes, Brazil, Dec. 14, 1921, 1402.

The above name seems to be the most acceptable one for this

distinct micro-form. The only other record of its occurrence from South America is from Surinam. It is known also from Porto Rico, Cuba and the Bahamas in the West Indies.

232. *UROMYCES ASCLEPIADIS* Cooke, Grevillea **5**: 152. 1877.

*Uredo Asclepiadis* Schw.; Berk. & Curt. Jour. Acad. Sci. Phila. **II, 2**: 282. 1853.

*Trichobasis Howeii* Peck, Ann. Rep. N. Y. State Mus. **23**: 58. 1873.

*Uromyces Howeii* Peck, Ann. Rep. N. Y. State Mus. **30**: 75. 1879.

*Uredo asclepiadina* Speg. Anal. Mus. Nac. Buenos Aires III. **12**: 316. 1909.

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*Asclepias curassavica* L. Lima, Peru, July 21, 1920, 776; Rio de Janeiro, Brazil, Aug. 13, 1921, 1035, Nov. 14, 1921, 1301; Cascadura, Rio de Janeiro, Brazil, Aug. 24, 1921, 1068; Sabara, Minas Geraes, Brazil, Dec. 2, 1921, 1362; Juiz de Fora, Minas Geraes, Brazil, Dec. 17, 1921, 1403.

A common species in North and South America and the West Indies. While in North America the species is known on a considerable number of species of *Asclepias*, most, if not all, of the South American collections are on the host listed above.

233. *PUCINIA OBLIQUA* Berk. & Curt.; Berk. Jour. Linn. Soc. **10**: 356. 1869.

*Puccinia Gonolobi* Rav.; Berk. Grevillea **3**: 54. 1874.

*Puccinia Cynanchi* Lagerh. Bol. Soc. Brot. **7**: 129. 1889.

*Puccinia Kunzeana* P. Henn. Hedwigia **33**: 230. 1894.

*Puccinia Philibertiae* Ellis & Ev. Bull. Torrey Club **22**: 60. 1895.

*Puccinia Oxypetali* P. Henn. Hedwigia Beibl. **38**: 129. 1899.

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*Oxystelma* sp. Sorata, Bolivia, May 3, 1920, 588.

*Philibertia* sp. Sorata, Bolivia, Apr. 15, 1920, 525.

*Unidentified asclepiad vine.* Reserva Florestal, Itatiaya, Brazil, May 9, 1922, 1831; Bosque da Saude, São Paulo, Brazil, March 22, 1922, 1668.

This characteristic micro-form is common throughout South and Central America as well as the Southern United States and the West Indies. It is probable that there are other synonyms which belong here among the numerous species of *Puccinia* described on members of this host family from South America.

#### SPECIES ON CONVOLVULACEAE

234. COLEOSPORIUM IPOMOAE (Schw.) Burrill, Bull. Ill. Lab. Nat. Hist. 2: 217. 1885.

*Uredo Ipomoeae* Schw. Schr. Nat. Ges. Leipzig 1: 70. 1822.  
*Coleosporium guaranticum* Speg. Anal. Soc. Ci. Argent. 17: 95.  
1884.

*Uredo Ipomoeae-pentaphyllae* P. Henn. Hedwigia 35: 252.  
1896.

*Peridermium Ipomoeae* Hedg. & Hunt, Mycologia 9: 239.  
1917.

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*Ipomoea acuminata* R. & S. Cascadura, Rio de Janeiro,  
Brazil, Jan. 12, 1922, 1471.

*Ipomoea purpurea* Lam. Santa Clara, above Lima, Peru,  
July 23, 1920, 784.

*Ipomoea* sp. Paineiras, Rio de Janeiro, Brazil, Aug. 17,  
1921, 1044; Friburgo, Rio de Janeiro, Brazil, Jan. 7, 1922,  
1466.

*Operculina glabra* (Aubl.) Choisy. Hacienda "La Florida,"  
Sur Yungas, Bolivia, May 28, 1920, 671; Pocos da Caldas,  
São Paulo, Brazil, Apr. 10, 1922, 1723.

235. PUCCINIA CONVOLVULI (Pers.) Cast. Obs. 1: 16. 1842.

*Uredo Betae Convolvuli* Pers. Syn. Fung. 221. 1801.

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*Convolvulus* sp. Therezopolis, Rio de Janeiro, Brazil, Oct. 6,  
1921, 1198.

This appears to be the first record of this rust in South America. There can be no doubt of the identification as a few typical teliospores were found, though the collection is mostly of uredinia.

236. PUCCINIA CRASSIPES Berk. & Curt.; Berk. Grevillea 3: 54.  
1874.

*Puccinia Ipomoeae* Cooke; Lagerh. Tromso Müs. Aarsh. **17**:  
61. 1895.

*Puccinia Ipomoeae-panduratae* Sydow, Monog. Ured. **1**: 323.  
1902.

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*Ipomoea polymorpha* Riedel, Arthur. Anvim, São Paulo,  
Brazil, March 15, 1922, 1636.

*Ipomoea*. Therezopolis, Rio de Janeiro, Brazil, Sept. 29,  
1921, 1170.

This characteristic -opsis form has been reported previously from South America from Argentina, Ecuador, Brazil and Colombia, usually under one or the other of the synonyms. It is a common form in Central America and the West Indies and in the Southern United States.

237. PUCCINIA DICHONDRAE Mont. in Gay, Fl. Chil. **8**: 46.  
1852.

*Puccinia Dichondrae* Berk. Jour. Linn. Soc. **13**: 173. 1872.

*Puccinia Berkeleyana* DeToni in Sacc. Syll. Fung. **7**: 717.  
1888.

*Puccinia munita* F. Ludwig, Zeits. Pflanzenkr. **2**: 133. 1892.

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*Dichondra* sp. Constitucion, Chile, Oct. 18, 1919, 129;  
Concepcion, Chile, Oct. 29, 1919, 145; Los Angeles, Chile,  
Oct. 30, 1919, 151; Cochabamba, Bolivia, March 12, 1920,  
404; La Paz, Bolivia, March 20, 1920, 441; Sorata, Bo-  
livia, Apr. 16, 1920, 528, Apr. 27, 1920, 578.

An *Aecidium* has been described by Neger and Hariot on *Dichondra*, which is assigned to this species by Sydow (Monog. Ured. **2**: 321. 1902). We have excluded the aecial names from the synonymy on the ground that this species is quite certainly a micro-form. The aecidium may be interpreted as the aecial stage of a correlated heteroecious rust or, since it is apparently systemic, it may prove to be an *Endophyllum*. Another possibility exists, however, which may be the true situation. I have in another publication (Mem. Torrey Club **18**: 58-64. 1931) discussed transitional -opsis forms and their probable relation to correlated micro-forms on the one hand and to heter-eu-forms

on the other. It is possible that there exists a form with an -opsis life history correlated with the micro-form *P. Dichondrae* and representing a transitional condition which has developed in the formation of the micro-form from the parent heter-eu-form.

238. ***Puccinia distinguenda* (Sydow) Jackson & Holway, comb. nov.**

*Aecidium distinguendum* Sydow, Monog. Ured. 4: 131. 1923.

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*Ipomoea fistulosa* Mart. Guayaquil, Ecuador, July 30, 1920, 794 (type).

The collection listed above bears abundant telia as well as aecia. The aecia agree perfectly with Sydow's description of *Aecidium distinguendum*. The telia are primarily epiphyllous, associated with the aecial clusters or on the same spots, and evidently from the same mycelium, small, round, early naked, jet black, pulverulent, ruptured epidermis at first noticeable. The teliospores are ellipsoid or oblong, 28–32 by 42–56  $\mu$ , rounded or sometimes obtuse above, rounded below, not constricted at the septum. The wall is dark chestnut brown, 3.5–4  $\mu$  thick, slightly thickened 5–6  $\mu$  at the apex and at the angles formed by the septum, evenly and finely verrucose. The pedicel is once to twice the length of the spore, colorless or slightly tinted next the spore, 10–11  $\mu$  at point of attachment, soon collapsing.

While the aecia somewhat resemble those of *Puccinia nocticolor* Holway, the teliospores are quite different. Pycnia were not observed with the aecia.

239. ***PUCCINIA LITHOSPERMI* Ellis & Kellerm. Jour. Myc. 1: 2. 1885.**

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*Evolvulus sericeus* Sw. Cochabamba, Bolivia, March 2, 1920, 360.

The above collection agrees with North American collections of this species. Arthur (N. Am. Fl. 7: 791. 1926) has reduced this name to synonymy and considers it the same as *Puccinia tuyatensis* Speg. The latter species, however, is described as

having the teliospore wall of uniform thickness. There is no evidence in the Arthur herbarium that the type of *P. tuyatensis* has been examined. On this account it seems best to record the species as above.

Spegazzini describes another species, *P. enecta* Speg. on this host genus, which may be the same as the above.

240. PUCCINIA OPULENTA Speg. Anal. Soc. Ci. Argent. **9**: 170. 1880.

*Aecidium Ipomoeae* Speg. Anal. Soc. Ci. Argent. **9**: 173. 1880.  
*Allodus opulenta* Orton, Mem. N. Y. Bot. Garden **6**: 195. 1916.

*Ipomoea* sp. Petropolis, Rio de Janeiro, Brazil; Oct. 18, 1921, 1226; Raiz da Serra, Rio de Janeiro, Brazil, Nov. 6, 1921, 1283; Bello Horizonte, Minas Geraes, Brazil, Nov. 30, 1921, 1349; Barbacena, Minas Geraes, Brazil, Dec. 12, 1921, 1387; Nova Friburgo, Rio de Janeiro, Brazil, Jan. 1, 1922, 1436; São Paulo, Brazil, Jan. 20, 1922, 1481; Juquery, São Paulo, Feb. 14, 1922, 1556; Tremembé, São Paulo, Brazil, March 6, 1922, 1616; Pocos da Caldas, São Paulo, Brazil, Apr. 8, 1922, 1714; Reserva Florestal, São Paulo, Brazil, May 9, 1922, 1826, 1830, May 14, 1922, 1851.

Evidently a common species in Brazil and Argentina. It has also been reported from Ecuador and from St. Thomas in the West Indies.

241. UROMYCES GEMMATUS Berk. & Curt.; Berk. Jour. Linn. Soc. **10**: 357. 1869.

*Uredo spinulosa* Dietel, Hedwigia **36**: 36. 1897 (not Sacc. 1891).

*Uredo Dieteliana* Sacc. & Sydow in Sacc. Syll. Fung. **14**: 395. 1899.

?*Uromyces giganteus* Dietel, Hedwigia **36**: 26. 1897 (not Speg. 1879).

?*Uromyces brasiliensis* Trotter, Ann. Myc. **2**: 533. 1904.

*Jacquemontia ferruginea* Choisy. Bosque da Saude, São Paulo, Brazil, Jan. 29, 1922, 1509; São Caetano, São Paulo, Brazil, March 8, 1922, 1617.

*Jacquemontia punctantha* Don. Guayaquil, Ecuador, July 31, 1920, 799.

*Jacquemontia* sp. Bello Horizonte, Minas Geraes, Brazil, Nov. 27, 1921, 1346; Campos do Jordão, São Paulo, Brazil, Apr. 28, 1922, 1786; Reserva Florestal, Itatiaya, Brazil, May 7, 1922, 1823; Guaruja, Santos, Brazil, July 18, 1922, 2015.

We have included here all the collections having thick walled spiny urediniospores and teliospores with greatly thickened apices. Some of our collections fit the species as described by Arthur in the North American Flora. Others, however, have much larger teliospores and fit the description and measurements of *U. brasiliensis*. The latter (as *U. giganteus*) was described with telia only, but has not been reported elsewhere than at the type locality. It would seem that this is a variable species and that the more northern collections have smaller teliospores than most of those from South America. Sydow found teliospores in the type collection of *Uredo spinulosa* which appear to fit our material well.

**242. *Uromyces vicinus* Jackson & Holway, sp. nov.**

II. Uredinia amphigenous, chiefly hypophyllous, scattered or gregarious in small groups, small, round, 0.2–0.4 mm. across, early naked, cinnamon brown, pulverulent, ruptured epidermis noticeable; urediniospores ellipsoid or obovoid, 19–22 by 24–28  $\mu$ ; wall thin, 1  $\mu$  or less, colorless or slightly tinted golden brown, closely and finely echinulate; pores obscure, 2 or 3, approximately equatorial.

III. Telia like the uredinia, blackish brown, compact; teliospores somewhat variable, ellipsoid, obovoid or oblong, 18–23 by 28–37  $\mu$ , rounded or obtuse above, rounded or often somewhat narrowed below; wall chestnut brown, thin, 1  $\mu$  or less, greatly thickened at apex, 6–12  $\mu$ , often somewhat lamellate, smooth; pedicel colorless or slightly tinted next the spore, short, deciduous.

*Ipomoea* sp. Petropolis, Rio de Janeiro, Brazil, Oct. 30, 1921, 1255; Barbacena, Minas Geraes, Brazil, Dec. 12, 1921, 1388; São João, São Paulo, Brazil, March 19, 1922, 1656, July 2, 1922, 1995; Tremembe, São Paulo, May 30, 1922, 1905; Juquery, São Paulo, June 12, 1922, 1961 (type); Alto da Serra, São Paulo, Brazil, June 14, 1922, 1964.

A characteristic species which differs from the preceding in both uredinial and telial characters.

#### SPECIES ON BORAGINACEAE

243. AECIDIUM BRASILIENSE Dietel, Hedwigia **36**: 35. 1897.

*Cordia discolor* Cham. Rio de Janeiro, Brazil, Nov. 13, 1921, 1297.

*Cordia obscura* Cham. Therezopolis, Rio de Janeiro, Brazil Oct. 2, 1921, 1185.

*Cordia* sp. San Francisco, Nictheroy, Rio de Janeiro, Brazil, Sept. 23, 1921, 1147.

A common species in southern Brazil. The report by Arthu, of this species from Trinidad is an error, as shown by an examination by the writer of all the specimens. They are *Aecidium Cordiae* P. Henn. The collection reported by Hennings from Yuremaguas, Peru (Ule 3242), was made the type of a new species *Aec. Lindavianum* Syd.

244. AECIDIUM CORDIAE P. Henn. in Engl. Bot. Jahrb. **17**: 491. 1893.

*Cordia curassavica* R. & S. Freguesia, Rio de Janeiro, Brazil, Nov. 18, 1921, 1318; Bello Horizonte, Minas Geraes, Brazil, Nov. 21, 1921, 1323.

*Cordia urticaefolia* Cham. Friburgo, Rio de Janeiro, Brazil, Jan. 6, 1922, 1463.

A characteristic species easily distinguished from the preceding by the thickened apices of the aeciospores. The type locality is usually given as Santo Domingo, but this is now considered an error for Haiti. The species is also known from Trinidad and at least two previous collections have been made in Brazil. Arthur (N. Am. Fl. 7: 635. 1924) lists *Aecidium brasiliense* Diet. as a synonym, but this is clearly an error.

245. AECIDIUM TOURNEFORTIAE P. Henn. Hedwigia **34**: 338. 1895.

*Tournefortia brachiata* DC. Juiz de Fora, Minas Geraes, Brazil, Dec. 17, 1921, 1404; Friburgo, Rio de Janeiro, Brazil, Jan. 2, 1922, 1445.

*Tournefortia grandifolia* Fresen. Bello Horizonte, Minas Geraes, Brazil, Dec. 4, 1921, 1364.

*Tournefortia* sp. São Paulo, Brazil, Jan. 22, 1922, 1489.

This species is common in southern Brazil and has also been reported from Porto Rico, Cuba and Panama.

246. ALVEOLARIA CORDIAE Lagerh. Ber. Deuts. Bot. Ges. 9: 346. 1891.

*Cordia cylindrostachya* R. & S. El Chaco, Prov. Sur Yungas, Bolivia, May 24, 1920, 642.

*Cordia* sp. El Chaco, Prov. Sur Yungas, Bolivia, May 24, 1920, 640.

Reported originally from Ecuador this interesting species is now known also from Colombia, Panama, Costa Rica, Guatemala, and from Jamaica.

247. *Puccinia Tournefortiae* Jackson & Holway, sp. nov.

0. Pycnia epiphyllous, closely aggregated in small groups of 3 to 8 on yellowish spots, noticeable, punctiform, dark reddish brown, globoid or depressed globoid, deep seated, 100 to 125  $\mu$  in breadth and height, ostiolar filaments short, not conspicuous.

III. Telia hypophyllous, usually forming a circular group 1-2 mm. across, with the pycnia in the centre on the opposite side of the leaf, confluent, early naked, compact, becoming pulvinate, golden brown, ruptured epidermis at first noticeable; teliospores elongate clavate or cylindrical, 22-29 by 56-100  $\mu$ , rounded or obtuse above, narrowed to pedicel below, not or slightly constricted, wall colorless or tinted golden brown, thin, 1  $\mu$  at sides, gradually thickened at apex, 6-12  $\mu$ , greatly thickened also at angles of lower cell just beneath the septum, smooth; upper cell usually broader and shorter than lower cell; pedicel short, colorless.

*Tournefortia fuliginosa* H.B.K. Sorata, Bolivia, May 4, 1920, 589.

This species appears to be somewhat like *P. tournefortiicola* Speg. In that species, however, the spores are considerably smaller. The teliospores in the above species germinate at once with a four celled promycelium. Three celled spores are not uncommon.

**248. *Uredo Tournefortiae* Jackson & Holway, sp. nov.**

II. Uredinia hypophyllous, scattered, small, round, 0.2–0.4 mm. across, early naked, pulverulent, light cinnamon brown, ruptured epidermis noticeable; urediniospores globoid or ellipsoid, 16–19 by 19–21  $\mu$ ; wall colorless or nearly so, 1–1.5  $\mu$ , closely and finely echinulate, the pores obscure.

*Tournefortia suaveolens* H.B.K. Huigra, Prov. Chimborazo, Ecuador, Aug. 3, 1920, 818.

Quite distinct from any *Uredo* previously reported on this host family. No pycnia or teliospores could be found.

**249. *UROMYCES DOLICHOSPORUS* Diet. & Holw.; Holway, Bot. Gaz. 31: 327. 1901.**

*Uredo pachystegia* Diet. Hedwigia 38: 257. 1899.

*Uromyces Tournefortiae* P. Henn. Hedwigia 47: 267. 1908.

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*Tournefortia psilostachya* H.B.K. Huigra, Prov. Chimborazo, Ecuador, Aug. 3, 1920, 813.

*Tournefortia sericea* Vahl. Juiz de Fora, Minas Geraes, Brazil, Dec. 18, 1921, 1412; Therezopolis, Rio de Janeiro, Brazil, Oct. 2, 1921, 1183; Friburgo, Rio de Janeiro, Brazil, Jan. 2, 1922, 1446.

*Tournefortia* sp. Reserva Florestal, Itatiaya, Brazil, May 10, 1922, 1841; Santa Anna, São Paulo, Brazil, May 25, 1922, 1881, 1882; Taipas, São Paulo, Brazil, June 10, 1922, 1950.

The type locality for this brachy-form is in Oaxaca, Mexico. This species is not infrequent in southern Mexico and is also known from Cuba and Porto Rico. It has not been collected frequently in South America. I find only the reference of the type collection of *U. Tournefortiae* which was made at Rio de Janeiro (Ule 2535) and the specimen on which *Uredo pachystegia* Diet. was based. The latter was reported as on Vernonia but according to Sydow (Monog. Ured. 4: 568. 1924) the host is *Tournefortia*.

250. TRICHOPSORA TOURNEFORTIAE Lagerh. Ber. Deutsch. Bot. Ges. 9: 347. 1891.

*Tournefortia* sp. Huigra, Prov. Chimborazo, Ecuador, Aug. 4, 1920, 834; Quito, Ecuador, Aug. 14, 1920, 892; Cuenca, Ecuador, Sept. 10, 1920, 967.

This remarkable micro-form is known only from Ecuador. The long hair-like columns of teliospores evidently adhere through the gelatinization of the spore wall. No evidence of a peridium has been noted. While no detailed study of the development of the sori has been made, it is quite evident that the spores are formed in chains. A careful study of crushed mounts of spore columns stained with cotton blue in lactophenol reveals the presence among the spores of additional, narrow, elongated binucleate cells, also with gelatinous cell walls. These are the "sterilen Zellen" of Lagerheim (l.c.). These narrow cells, which are about one fourth the diameter of the spores, appear to be connected with them by greatly elongated extensions of the gelatinous spore wall. Their presence may be demonstrated even after the spores have germinated, by the deeply stained contents. It seems reasonable to interpret these elongated cells as intercalary cells.

The spores germinate by the development of internal transverse walls which divide the spore into four cells. Rather stout sterigmata develop from each cell. These are short in the spores which occur on the outsides of the spore column, but are much longer from the internal spores. The basidiospore is obovate 11–13 by 17.5–18.5  $\mu$ .

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TORONTO, CANADA.

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